

ECOTOX

ECOTOXicology Database System

AQUIRE Coding Guidelines

Prepared for

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Mid-Continent Ecology Division (MED)
Duluth, Minnesota

By

Computer Sciences Corporation
Duluth, Minnesota 55804
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REVIEW OF LITERATURE

A. OVERVIEW OF ECOTOX

1. Introduction to Review of Literature

The data elements included in ECOTOX encompass standard test parameters typically reported within a publication. Each database record contains information about the exposure and test conditions. Specific parameters include the test chemical, species, and endpoint or effect concentration.

The included literature is identified through standardized bibliographic retrievals. Each publication is evaluated and the applicable data is encoded by trained literature reviewers. The data encoded are evaluated according to existing standard test methods such as those from the American Society for Testing and Materials (1996), Code of Federal Regulations (1992), and the American Public Health Association (1992). Each test included in ECOTOX is assigned a documentation code that indicates the amount of supporting methods and results documentation available in the original scientific publication.

Note: Each publication included into the AQUIRE database must meet the five minimal criteria for acceptance (i.e. chemical, species, concentration, duration and effect). If the paper is missing one or more of these criteria ECOTOX does not search other sources to obtain the missing data piece(s). Sources such as author communications and referencing another work to obtain one of the five criteria is allowed for specific risk assessment/criteria projects (e.g. EcoSSL or CAD) and must be specified by the EPA Database Coordinator.

2. Literature Reviewer Training

Training Sequence

The training sequence is designed to develop consistent, accurate, and versatile literature reviewers. This is accomplished through an intensive period of literature review, interactive quality assurance procedures, and consultation with other ECOTOX database personnel.

The scope of the six month intensive training period encompasses the following areas:

- endpoint toxicity test review (one month);
- effect only toxicity test review (two months);
- bioconcentration study review (one month);
- field study review (one month); and
- in-depth training within the areas listed above (one month).

The personnel available to support the reviewer include the data coordinator and trained ECOTOX reviewers. The following documentation and materials are used for training:

- ECOTOX Standard Operating Procedures (2001, www.epa.gov/ecotox under "download files");
- Fundamentals of Aquatic Toxicology (Rand (Ed) 1995);
- American Society for Testing and Methods; (ASTM, 1996);
- Selected toxicity literature publications; and
- AQUIRE and TERRETOX coding sheets.

The reviewer initially reviews the ECOTOX Standard Operating Procedures: Coding Guidelines, applicable publications listed in the reference section for each of the databases, applicable US EPA Standard Evaluation Procedures and ASTM guidelines. The primary emphasis is to understand the minimum criteria that characterize acceptable toxicity tests. These criteria must be reported in the toxicity publications selected for review in order to qualify for inclusion in the ECOTOX database. The acceptance criteria are:

- Name of the test **chemical**;
- Name of the **test organism**;
- **Effect** of the test chemical on the organism;
- Test chemical **concentration** or application rate;
- Test **duration** (except for abstracts and non-English publications).

The secondary emphasis is to develop the ability to distinguish between exposure types (lethal, sublethal, bioconcentration). The reviewer is trained to recognize whether standard methods are reported for test methodologies and for the test endpoint. The reviewer is also trained to identify tests which are not applicable to ECOTOX.

Once the general introductory materials are read, the standard training guidelines introduce the reviewer to each category of toxicity literature. Information specific to areas of acute, chronic and bioconcentration literature is discussed in subsequent sections of this chapter. The guidelines can be tailored to the specific areas of expertise and strengths that each person brings to the project. Three primary elements are emphasized in each component of the training sequence. The standard training sequence is:

1. Example review: Examination of previously encoded toxicity literature. The trainee reviews between 5 to 10 toxicity publications and compares each with its associated pre-completed coding sheet.
2. Independent review: The trainee independently reviews a minimum of 10 to 20 toxicity publications. All 10 - 20 reviews are quality assured via a review of the publication and coding by the data coordinator. Inconsistent coding practices are resolved with the trainee. The trainee continues to review additional toxicity publications and the level of QA decreases from 100 percent to 10 percent as the reviewer's consistency and proficiency increase.

3. Measure of proficiency: Established ECOTOX quality assurance procedures require a close review of all reviewed publications by the data coordinator to ensure accurate reviewing is consistent with current test methodologies and SOPs. All discrepancies identified are noted by the data coordinator and discussed with the trainee.
4. A full time reviewer begins the training sequence reviewing 20 publications per month. This amount increases until a level of 35 publications per month is attained. The average time estimated per review at the beginning of the training sequence is 1.5 hours per publication. The time should decrease to one hour per publication. A part time reviewer's training expectations will be decreased accordingly.

Measures of Competency for Trained Reviewers

The quality assurance process is an ongoing component of literature reviewing. Emphasis is placed on quality assurance during the initial collaborative training period, during the 10 percent replicate review process, and through consultation with publications in the field of aquatic toxicology. As part of this process, consistency and concurrence between the document abstractors is attained.

The ten percent replicate review process assures data integrity and promotes routine evaluation of coding practices. Through this training process, strengths and weaknesses in the data abstractor's expertise are identified and specific programs are established to enhance expertise where needed. Such programs include consultation with ECOTOX staff, toxicology publications and the EPA Database Manager, as needed. Evaluation of replicate reviews, which is performed on 10% of all coded references, is used to flag and correct any major discrepancies between replicates. In addition a screening of all completed coding sheets to ensure consistency and completeness prior to data entry is required. Parameters routinely screened include water chemistry, test organism descriptors, calculated endpoints and total test numbers.

Steps in the Quality Assurance Process

1. Ten percent of the reviewed articles from each abstractor are randomly identified by the data coordinator. Information concerning the number of publications is entered into a Lotus 1-2-3 file, maintained on the data coordinator's computer. The spreadsheet tracks the QA process and calculates the percent of the publications subjected to quality assurance for each reviewer (Table 1). The original reviewer's code sheet for the chosen publication is placed in the "Double Review and QA" file folder maintained by the data coordinator. An "ECOTOX 10% Tracking Form" sheet is maintained in the folder and filled out as articles are received (Attachment 1.). The spreadsheet file is also updated.

Table 1. ECOTOX 10% TRACKING SHEET EXAMPLE

| Date Rec | Doc # | Tot Rec'd | # QAed | 2nd Rev | 2nd Comp | Coord | Complete d |
|----------|-------|-----------|--------|---------|----------|-------|---------------|
| 11/30/93 | 5342 | 8 | 1 | JACKY | 11/30/93 | ANNE | 01/15/94 |
| 12/14/93 | 6808 | 10 | 1 | AMY | | | |

2. The publication is given to a second reviewer for independent review. After completion of the second review, the data coordinator gives the coding sheets and paper to the EPA Database Manager who compares both reviewer's coding sheets, documents the differences (if any) between reviewers, archives the information on the "ECOTOX 10% Replicate Review" form (Attachment 1.), then returns the form to the reviewers for comment. The reviewers note discrepancies by either agreeing with the EPA Database Manager's comments or expressing their differing opinions on the form. After the replicate review form is returned to the EPA Database Manager, discussions are held with both reviewers to resolve any remaining differences. Discrepancies due to differences in interpretation are resolved by the EPA Database Manager. Errors caused by incomplete Coding Guideline documentation are identified and modifications are made to the document.
3. Upon completion of the review process, the data coordinator checks to make sure the original reviewer's coding sheet contains the correct data, notes completion date on the "ECOTOX 10% Tracking Form" and in the spreadsheet, and forwards the coding sheet to data entry. The ECOTOX 10% Replicate Review forms are filed with the double review coding sheets in the ECOTOX Reviewed files.

3. General Coding Information

Overview

ECOTOX is comprised of two databases - AQUIRE and TERRETOX. The AQUIRE database is comprised entirely of aquatic data whereas the TERRETOX database is comprised of terrestrial data. These databases were developed independently but are merging together under the ECOTOX framework. Across the two databases, the common data elements for each test contained in ECOTOX are grouped by chemical, organism, exposure conditions, and effects. Test chemical parameters describe the toxicant and any associated carrier; the CAS registry number; and the grade, purity and/or composition. The test organism parameters include the Scientific name, a species number and lifestage, source, and/or characteristics of the organism. The test conditions identify the test location; exposure type, time, and conditions; and any control parameters. Effect and endpoint parameters consist of codes to define lethal, sublethal, or residue effects and/or endpoints.

The corresponding chemical concentration or dose is reported for both exposure and observation concentrations, if reported. Available data are extracted from the text, tables, and graphs of each publication.

Based on the information coded for the preceding categories, a documentation code is calculated for each piece of data in ECOTOX. The documentation code provides an index of the completeness of methods documentation and results presentation in the original publication.

The following sections are designed as an overview of the guidelines for reviewers. The information presented in this section identifies the common and unique attributes of each database. Each section heading corresponds to a data element (if the data element is unique to one or two of the databases, this is noted following the section heading). The unique attributes of each database are described in the specific coding guidelines for AQUIRE and for TERRETOX. Any exceptions from these guidelines must be authorized by the EPA Database Manager and subsequently documented in these guidelines.

Coding Practices

This section provides an overview of the general coding practices used for the ECOTOX database. These practices have been devised to ensure accuracy and consistency in transcribing data from the original publication to the final data file.

- A unique coding sheet is used for each of the independent databases - AQUIRE and TERRETOX.
- Each reported test exposure (or in some cases each unique endpoint) requires a separate line on the coding sheet. If many tests are reported that are conducted under similar conditions, ditto marks are placed in the field or remarks area where the information is identical to the line above.
- Endpoints, effect or exposure concentrations/doses, control data and exposure times reported in graphic format are coded.

Data extracted from graphs are presented as range or <, > values, unless an exact value is clearly presented. If the format of the graph does not allow extrapolation, the availability of such data is noted in REMARK, i.e., "/control data graphed/". Data extracted from a graph must be accompanied by a comment in the REMARK field "/from graph/". When there is a discrepancy between data presented in the text or table and data presented in a graph, the paper is to be forwarded to the EPA Database Manager for a final determination of which data point will be included in the database.

- To ensure completeness and accuracy, if information is unavailable for a coding field, the field must still be completed using either NR (not reported) or occasionally, NA

(not applicable).

- To ensure accuracy in transcribing data values, all numbers between zero and one should be reported with a zero preceding the decimal point (e.g., 0.5 not .5). Periods are only used to represent a decimal point, never an abbreviation.
- To ensure consistency as well as accuracy, report the significant figures as the author reports them. Do not add or round off numbers. Report only the actual values, do not code variance information (e.g. +/-).
- When coding numbers do not use commas. They can be mistaken for decimal points or numbers.
- Use "per" or a colon (:) instead of a slash (/) to designate ratios. Reserve the slash for designating remarks or units.
- The REMARK field is a text field which contains additional information about a coding field. The REMARK field is used when the information necessary for coding a field does not fit in the space provided. A complete list of remark identifiers is documented in the appendices for each of the databases.
- When making a remark, use the appropriate codes from the ECOTOX code list. If a code does not exist for a certain chemical, enzyme, devise, technique, etc., do not use the author's abbreviation. Write out the full name of the term and submit to the ECOTOX staff to determine if a new code should be created.
- All coding sheets with the same reference number and the same chemical CAS number are stapled together in the upper left corner.
- Coding sheets are generally double sided. If any tests are coded on the back of the coding sheet, the "continued on back" text located in the lower right corner of the coding sheet is highlighted in yellow.
- When a reviewer has completed the review of a paper, they must write their last name preceded with "R=" on the bottom center of the paper.

B. AQUIRE CODING GUIDELINES

A unique coding sheet is used for each of the independent databases - copies of the AQUIRE coding sheets are located at the end of this section. For AQUIRE, field (natural and artificial) tests are coded on the AQUIRE Field Coding Sheet; all other studies are coded on the AQUIRE Lab Coding Sheet.

1. Quality Assurance Parameters

QA Date/Initials

The person conducting the first Quality Assurance Check enters the date of the QA check and their initials.

Publication Reference Number, Author, Year

The Reference Number (Ref #) is the unique number which identifies a particular publication. This number, assigned by the data entry program, provides the link between the data entered and the original publication. On the coding sheet, enter the reference number located in the upper right-hand corner of the hard copy of the publication, the last name of the first author, and the publication year. For abstracts, use the publication year of the abstract source.

Total Tests

The total tests encoded for a publication are recorded by the reviewer. The total test number equals the total number of individual effect records that are coded for each publication.

Reviewer/Date

The reviewer's last name is written here. The date on which the publication was reviewed should be entered in the format of month/day/year.

2. Test Chemical Parameters

AQUIRE is catalogued by the toxicant tested using the Chemical Abstracts Service (CAS) registry number. If a CAS registry number is not available through standard sources the toxicity data cannot be included in AQUIRE. Additional toxicants not included in AQUIRE are water chemistry effects (e.g., pH), complex effluents, and chemical mixtures.

Chemical mixtures may be interpreted broadly. For example, if a pesticide is a mixture of two active ingredients, each may have a separate CAS number. If the formulation has a CAS number, the chemical reported for AQUIRE is the formulation. If the exposure is based on two metal compounds but the effect is based on one ion, e.g., copper sulfate and copper chloride and Cu is the toxicant, code copper as the test chemical and report the two compounds in chemical CHARACTERISTICS.

For *in situ* exposures where the exposure is by default an exposure to a chemical mixture; code residue effects or endpoints (BCF) only. No other effects or endpoints are strictly attributable to a single chemical in the same way as a residue concentration. Data for chemicals in the mixture with reported water concentrations and residue effects should be coded. The only situation in which a mixture exposure can be coded is when an *in situ* field study is conducted. The test organisms must be transplanted from a clean source and caged in the polluted source. The duration and concentration must be provided. When the author provides only the species age for the duration, it is unacceptable to code.

Effect responses from exposures to hormones (e.g. estradiol, testosterone) are included in the database. There are studies where the hormone is administered as a toxicant to observe the andro/estrogenic effects.

Studies involving carbon dioxide (CO₂) or ozone (O₃) as the toxicant are not coded into the AQUIRE database.

Nutrients such as phosphorus, nitrogen, potassium are coded for AQUIRE if the exposure system is dosed rather than an ambient exposure. For example, code phosphorus as an exposure chemical if, in the given paper, all of the following are true:

- The phosphorus was added to the ecosystem in a direct discrete manner, i.e., code *"nylon mesh bags of Ca(H₂PO₄)₂ placed in streams at beginning of test"*, do not code *"system may have received added phosphorus in overland runoff due to fertilizers used in nearby agricultural operations"*. Aerial applications are acceptable if the other conditions are met.
- The concentration in the water should be measured, or at a minimum, the application rate should be available. Application rate may be calculated using the flow volume and the phosphorus-containing compound's dissolution rate.
- The effects of the phosphorus are tested on a biological test organism; water quality or chemical-fate only papers are not coded.

Effect responses from exposures to humic acid only are not coded for AQUIRE. Humic acids are any various complex organic acids obtained from humus which are insoluble in acids and organic solvents. However, tests that include an exposure with a toxicant and humic acid should not be interpreted as a mixture. The humic acid information should be coded into the ORGANIC CARBON field if the concentration is given and "Humic Acid Efcts" should be coded in the OTHER EFFECTS field.

Example: The toxicant is Copper and Humic acid is added at 10 mg/l.
Code the concentration of Humic acid in the ORGANIC CARBON field as 10 mg/L HA.

Chemical Name (TEST)

Record the chemical name as it is reported in the publication; however, long chemical formulas or names need not be coded if a common name is provided. For common names, record common name in both the TEST field and the CHARACTERISTICS field; when the CAS number is entered into the system the 9CI Preferred Name will be assigned automatically. The TEST field on the coding sheet is used for the convenience of the encoder in assigning the CAS number. If several names (e.g., trade names, synonyms) are used, note the other names and formula in parenthesis after the recorded chemical name.

The CAS number is assigned by locating the chemical name in the chemical card file or in the online index file (ECOCHM). If the chemical name is not in the chemical card file or ECOCHM, write "No" near the CAS NUMBER field to clearly identify that verification is needed. The coding sheet will be referred to ECOTOX staff for CAS number verification as part of the quality assurance process.

Chemical Grade (GRADE)

Record relevant chemical grade information in the GRADE field. (refer to ECOTOX Appendix B).

Chemical Purity (PURITY)

Record the numeric percentage information about the purity or active ingredient of the chemical in the PURITY data field (e.g., if the author reports 97% purity, 97 would be entered into this data field. PU for purity would be entered into the FORM data field (see CHEMICAL FORMULATION).

Chemical Formulation (FORM)

Record the chemical formulation code for the chemical reported. If there is more than one formulation code enter the code most closely related to the chemical purity, and enter the rest in the CHARACTERISTICS field. (refer to ECOTOX Appendix C)

Chemical Comments (CHARACTERISTICS)

Record relevant and specific chemical information, such as trade names, common names, isomers, or extra formulation codes, but do not code lot numbers or product numbers (usually taken from chemical catalogs). There are times when you will record the chemical name in both the TEST field and the CHARACTERISTICS field. This occurs most frequently for pesticides where the common or trade name is very simple while the chemical nomenclature is very complex. The purpose, during reviewing, for the name in TEST field is to assist the reviewer in assigning a CAS number; during data entry the name is replaced by a stored 9CI Preferred Name. The common name coded in CHARACTERISTICS remains available for user access.

Radiolabel (RADIOLABEL)

If a radiolabeled chemical is tested, record the isotope, according to the ECOTOX Appendix D codes, in the RADIOLABEL field. When the specific isotope is not reported, the field should be coded with a slash ("/") and noted in the REMARK field (RADIO/no isotope reported//). When both radiolabeled and unlabelled test chemicals are used in a test, report the radiolabel isotope and code "labelled and unlabelled" in CHARACTERISTICS.

CAS Number (CAS NUMBER)

The Chemical Abstracts Service Registry Number of the toxicant is recorded in the CAS NUMBER field. A standardized identification number and name for each chemical recorded in the database is used for consistency. Toxicants included in the ECOTOX database are assigned a CAS registry number and are referred to by the Ninth Collective Index (9CI) standard nomenclature. The CAS number and 9CI name are stored in a chemical card file and in an online index file (ECO-CHEM) which is available electronically for screening CAS numbers and chemical names used in ECOTOX. If a hydrated form of a chemical is used in the paper, record the hydrated form as reported by the author in the TEST field. However, record the CAS Number for the non-hydrated form of the chemical in the CAS NUMBER field.

| | | | |
|----------|-------------------------|---------------------------------------|--------------|
| Example: | Chemical cited in paper | CuSO ₄ * 5H ₂ O | CAS# 7758998 |
| | <u>TEST</u> field | CuSO ₄ * 5H ₂ O | |
| | <u>CAS NUMBER</u> field | CAS# 7758987 (CuSO ₄) | |

Solvent Chemical (SV)

If a solvent carrier is used in the test, the solvent chemical fields are coded with the Solvent Chemical (SV), GRADE, PURITY, FORM, concentration (in CHARACTERISTICS) and CAS NUMBER. The CAS numbers for common carriers are listed in ECOTOX Appendix A.

Occasionally two or more separate carriers or solvents are used. If the publication reports the ratio, include this information in the CHARACTERISTICS field. If the carrier or solvent is used for different test chemicals but the use is not specifically described in the publication, code "as needed" in the CHARACTERISTICS field.

If an author reports the concentration of the solvent used during the preparation of the stock solution and reports the concentration of solvent that the organism received, the concentration that the organism received is reported in the solvent CHARACTERISTICS field. If the concentration reported for the solvent in the control differs from the concentration dosed to the organism the concentration that the organism received is reported in the solvent CHARACTERISTICS field.

If a carrier was not used, report as NR. Buffers used to control the pH of the test are not coded. Acids or bases that are added to change the pH of a solution in order to enable a metal to stay in

solution are not coded. Dietary feed content is not coded.

Occasionally, an author will present results data related to effect of the solvent on the test organism. If a test concentration and results are reported for the solvent and there is a separate clean water control, the solvent data should be coded on a separate coding sheet.

If an author states that all solvent was evaporated prior to the study or if a column coating procedure is described, the solvent is assumed to not be incorporated into the study and should be coded as NR in the S/V field.

For instances where a chemical carrier does not have a CAS number and it is determined by the chemical verification staff that it will never have a CAS number, the solvent will be entered in the CHEMICAL COMMENTS field of the Chemical Name (TEST).

If the solvent does not have a CAS number and it is in the verification process, data entry will enter NR in the S/V field and enter a remark of CARRIER/solvent name and characteristics//. When the CAS# is verified a search is made in the remarks for the chemical name and those records found are modified.

Example: The solvent used is Atlox at 0.05 ul/l. There is no CAS# and it is in the verification process. Entered into REMARKS: CARRIER/ATLOX, 0.05 UL/L//

If an author presents results in which the test organism is exposed to multiple chemicals, it is important to determine if the two chemicals constitute a mixture or if one of the chemicals is being used as a carrier or solvent. A carrier is defined as an agent (other than water) in which the test chemical is mixed to make it miscible with the dilution water before distribution to test chambers (Rand, 1995). If the other chemical is not a carrier, "mixture" would be noted in OTHER EFCT.

Water should not be coded as a solvent. A solvent is defined as an agent (other than water) in which the test chemical is mixed to make it miscible with dilution water before distribution to test chambers. Solvents or carriers are used in toxicity tests where the concentrations of the test chemical are extremely low and a very small amount of test material must be added to the test chambers. (Rand, 1995)

3. Test Organism Parameters

Species (LATIN NAME/SPECIES NUMBER)

Record the species Scientific name as reported in the publication. A unique number is assigned to each ECOTOX species to aid in storage and retrieval. Reviewers locate the number for each test organism from the CRITTERS species file and record this number on the coding sheet. If the species name reported in the publication is a synonym of a verified species, record the name from the publication, draw a line through it and record the verified species name along with the species number. If the species is not on the verified name list, write "No" near the Scientific

name to clearly identify that verification is needed. The coding sheet will be referred to ECOTOX staff for species verification as part of the quality assurance process. Refer to Species Procedures for additional information about the species data file and verification procedures.

Field studies may report results for a target community (e.g. benthic macroinvertebrates) or for an entire enclosed ecosystem (e.g. system-level primary productivity or respiration). If a community of organisms was tested, be as specific as the author is about the species grouping.

Lifestage (LIFESTAGE)

Report the specific lifestage for the test organism at the beginning of exposure, as reported in the paper (see Appendix F for lifestage codes). Record as 'NR' if the information is not reported in the publication. The following are examples of data coded in this field:

- a) The author reports adults are tested

Lifestage: AD

- b) The author reports exponential growth phase algae are tested

Lifestage: EX

Tests in which eggs are initially exposed, and the exposure continues through adulthood to the first generation etc, are represented as "EG" in LIFESTAGE and the stage of the organism is recorded in the EE REMARK field for the results reported.

Example: Exposed eggs resulting in mortality of fry

LIFESTAGE: EG

EFCT: MOR

MEASURE: MORT

EE REMARK: Fry

Age (AGE) and Age Unit (AGE UNIT)

Report the age and age unit for each test organism at beginning of exposure, as reported in the paper (see Appendix I for associated time units). The age may be a development stage if no specific time is reported. For example: The author reports that 4th instar larvae were used in the study. The following is coded:

Lifestage: LV

Age: 4

Age Unit: inst

Record as 'NR' if the information is not reported in the publication.

Organism Comments (ORGANISM CHARACTERISTICS)

Report any general information provided about the initial condition of the test organism that is not coded in the LIFESTAGE and/or AGE and AGE UNIT fields. This includes information for both the control and test organisms. Organism comments include information such as weight, length,

developmental stage, sex, type of culture (eg., axenic) and/or initial cell concentration (e.g. 1 E + 3 cells/ml) to describe the organism being tested. Each piece of information is separated by a comma. The value and range, if reported, are recorded for each available parameter (e.g. 3 (2-4) g). However, deviations are not coded (e.g. 33 +/- 4 mm is coded as 33 mm). Record strains, hybrids or taxonomic groupings, if reported. List individual species Scientific names when 3 or fewer species are included within a grouping; when more than 3 individual species are included within a grouping, code as "# species".

Species = Plankton (#706)

ORG CHARACTERISTICS = *Daphnia magna*, *Daphnia pulex*, and *Bosmina* sp

ORG CHARACTERISTICS = 4 zooplankton species

Standard terms used for recording organism length include standard length (SL), (e.g. 3.1 cm SL), total length (TL), fork length (FL), carapace length (CL), carapace width (CW), wet weight (wet wt), and dry weight (dry wt).

If the paper states that the organisms tested are both male and female, this characteristic does **not** go into the ORGANISM CHARACTERISTICS field, because a sample assumes both sexes. However, if only one sex is tested, then the sex is coded using the terms "male" or "female".

If a paper reports results using organisms from a polluted source AND organisms from a non-polluted source, only the non-polluted source test results are coded. No mention of 'non-polluted' needs to be presented in the ORGANISM CHARACTERISTICS field, but 'polluted organisms' should be entered into OTHER EFCT data field. If data are presented for organisms from a polluted source and no other concurrent data with organisms from a clean source are presented, the reviewer should code the test results, but enter 'polluted organism' in the ORGANISM CHARACTERISTICS data field.

If a paper reports that the organisms used for testing were diseased, "Diseased Organisms" is entered into the EXPERIMENTAL DESIGN field. If the study was conducted on diseased organisms compared to non-diseased organisms, the data from the non-diseased organism test is coded, but the data from the diseased organisms is not. In this case, "Diseased Organism Test" is entered into the OTHER EFCT field.

If a paper reports that the organisms used for the measurements were dead and it is unknown how long the organisms have been dead, do not code this data, but enter "dead organisms" in OTHER EFCT. If the study was conducted on dead organisms compared to living organisms, the data from the living organism is coded but the data from the dead organism test is not coded. In this case, dead organism is entered into the OTHER EFCT field.

If some of the organisms tested are fed differing amounts and/or some of the organisms are not fed during the study and the authors are comparing the data (e.g. 10 organisms fed, 10 organisms not fed and/or some organisms fed 10 pellets and some organisms fed 5 pellets), all data are coded, "Fed", "Not Fed" or food amount is coded in the EXP DESIGN field, and "Feeding Efcts" is coded in the OTHER EFCT field.

The source of the organism does not need to be coded in the ORG CHARAC field unless the author(s) compare results based on the source of the organisms. "Organism source effects" should also be noted in the OTHER EFCT field.

Example: ORG CHARAC: Hatchery-reared
OTH EFCT: Organism Source effects//

Control Type (CNTL)

The type of test control(s) used in the study is reported in this field. Control information for the reported effect may be presented in the text, in a graph, or in table format. ECOTOX reviewers do not make assessments whether the controls were satisfactory or insufficient (e.g., were replicates run, did control organisms die), but simply document whether the author(s) present information that a control was used. When author's state that controls were similar to treatment with the exception that no chemical was added, and within the same paragraph they describe using solvent in all treatments, a solvent control should be interpreted. (refer to ECOTOX Appendix M for control type codes and definitions.)

When statistical comparisons are presented for multiple controls (e.g., statistics in relation to a standard control and statistics in relation to a solvent control), code **M** in the CONTROL TYPE field and note the appropriate control type used for the coded statistics in EE REMARK. (e.g., stats based on solvent control//) The data related to the solvent control is coded preferentially over the standard control data and should be noted as such in the EE REMARK field.

When multiple controls are reported by the author, code "M" in the CONTROL TYPE field and list the various controls reported in a remark.

Example: Author reports the use of clean water control and a solvent control

CODE: CONTROL TYPE: M/ REMARK: CONTR/C,V//

4. Test Condition Parameters

Media Type (FW, SW)

The type of water or media is coded in the FW,SW field. Freshwater (FW) tests include 1) laboratory tests conducted in freshwater, reconstituted water, distilled water, or tap water or 2) field tests where the organism habitat is exclusively freshwater. If a salinity value of <4 ppt is reported and the paper does not specify whether it is fresh or saltwater, it will be coded as a freshwater test.

Saltwater (SW) tests include 1) laboratory tests conducted in natural or artificial seawater, brackish water, or estuarine water or 2) field tests where the organism habitat is exclusively saline.

If a determination cannot be made regarding the use of either freshwater or saltwater, an NR (not reported) is recorded.

For example, a natural field study (FieldN) is an experiment conducted outdoors in a natural setting in which the test organisms are confined via an enclosure of some type (cage, fencing, plot lines) or sampled in the wild. Field exposures are exposed to uncontrolled variables such as weather. An important component for classification as natural is that the setting includes a bottom substrate as well as a community of representative organisms. Outdoor studies conducted in a simulated environment are coded as an artificial field study (Field A) study. Such studies include organisms isolated from their natural environment while still out of doors, e.g. earthen or concrete ponds without sediment or with only one representative species.

Laboratory tests are conducted under indoor controlled laboratory conditions (light and temperature regulated). If the location or setting cannot be determined from the publication code as Not Reported (NR). For AQUIRE, field (natural and artificial) tests are coded on the AQUIRE Field Coding Sheet; all other studies including tests not specified field or lab, are coded on the AQUIRE Lab Coding Sheet.

Study Type (STUDY TYPE)

For laboratory exposures, the study type is used to identify field simulation studies. For example, indoor mesocosm or microcosm studies should be noted as such in the STUDY TYPE field. If information about the study type is not reported, leave this field blank.

For field exposures record the study type as reported by the author in the STUDY TYPE field. Examples of field study types include, but are not limited to, exposures with caged organisms or conducted in a mesocosm, microcosm or enclosure. If information about the study type is not reported, leave this field blank.

The valid codes for this field are:

ARTIFICIAL
CHANNEL
ENCLOSURE
LITTORAL
MESOCOSM
MICROCOSM

Experimental Design (EXP DESIGN)

This field is used to code additional study information. For field tests, report exposure system dimensions (e.g. pond or lake depth, cage or enclosure size), type of artificial substrate and physical or chemical water chemistry parameters.

EXP DESIGN: 3 ha polyethylene lined pond// EXP DESIGN: 4 x 4 m cage//
 EXP DESIGN: sediment// EXP DESIGN: humic acid//
 EXP DESIGN: Instant Ocean®// EXP DESIGN: Sinking Cr water//

For laboratory studies, information about media and test chambers is coded if one of the purposes of the study is to compare results observed under differing test conditions (e.g., pH, temp, humic acid, sediment) or if commercial media types (e.g. Instant Ocean®) were used in the study. If one of the purposes of the study is to compare experimental effects (pH, temp, sex) in addition to toxicant effects, report the additional effects in the OTHER EFCT field. (Refer to ECOTOX Appendix V for a list of keywords)

Example: OTHER EFCT: pH efct//

Information about the dilution water is provided if needed to distinguish one test scenario from another, e.g. natural waters from three different ponds, sites on a river, locations in a sea. Tests with differing dilution water are coded as separate lines of data; it is not acceptable to combine tests by effect or water chemistry variables across differing dilution water test scenarios.

If an organism is pre-exposed to another chemical and this is the only information that can be coded, the chemical that is associated with the observed effect is coded and "preexposure with X" is noted in the EXP DESIGN field.

On occasion, an author will note that organisms were sampled during the course of a study for analysis. "Sub-sampled" should be noted in the EXP DESIGN field.

Example: Author tests organisms for residue and mortality over a 30 day study. At day 5 and 10, several organisms are pulled from the study for residue analysis. At the end of the test (30d) the mortality is reported. "Sub-sampled" should be coded in EXP DESIGN field since these sub-sampled organisms may or may not be included in the final calculation for mortality.

When coding field exposure publications, additional related coding parameters that do not get coded in the STUDY TYPE, HABITAT CODE, and SUBSTRATE fields are added to the EXPERIMENTAL DESIGN field. When adding field exposure information to the EXP DESIGN field, make sure to precede all data added with the appropriate field name code.

Example: EXP DESIGN: HAB/suspended mesh bags//

5. Test Result Parameters

Toxicity test results for the AQUIRE database are represented by a combination of the ENDPOINT, TREND, EFFECT, RESP SITE, EE REMARK, MEASURE, EFCT%, SIG, LEVEL, CONC and BCF fields.

Toxicity test results for AQUIRE are primarily reported for observations taken during the chemical exposure; however, when results are reported *only* for the period of time after the exposure

(moved to clean water), ie. recovery or delayed effects, this type of result is noted by using a "~" in conjunction with the endpoint/effect code, e.g. ~MOR for a delayed mortality effect.

Figure 1. shows the hierarchy of coding tests in AQUIRE.

Endpoints always require a discrete line. For data not reporting an endpoint, at least one separate line is coded for each measurement from either a unique experimental design or within one design scenario for statistically defined data points. .

Food chain effects or endpoints are coded for organisms at the first level of exposure. Subsequent levels of exposure are not coded, but are noted in the OTHER EFCT field, e.g. Other Effects: food chain study//.

Endpoint, Effect, Measurement and Statistics sections have further description and examples.

The following sections provide a brief description for each of these fields, followed by guidance for coding information from the publication for each of the fields.

Endpoint (ENDPOINT)

For the purposes of AQUIRE, an endpoint is the quantification of an observed effect obtained through statistics or other means of calculation for the express purpose of comparing equivalent effects (e.g., LC50). ECOTOX Appendix T identifies and defines the ECOTOX endpoint codes. The endpoint field will be coded as NR if the author does not report or define an endpoint or there is no companion data point.

Endpoint information is coded into AQUIRE if it is reported by the author, if the author's definition of the effect is equal to AQUIRE endpoint definitions, or if the data point is a companion endpoint to a LOEC, NOEC and/or MATC. "Companion endpoints" are endpoints assigned by the reviewer when the statistical results follow a clear concentration-response pattern and the author reports a NOEC, LOEC or MATC but fails to report the "companion endpoint". For example, when an author reports a NOEC and does not specifically define the lowest statistically significant effective concentration as a "LOEC", the data point is coded as a LOEC in AQUIRE by the reviewer. Similarly for reported LOECs without NOECs, NOEC/ LOECs without MATCs and MATCs without NOEC/LOECs.

If within a study an author reports a NOEC and/or LOEC endpoint for one of the measurements in the text but does not specify the endpoints for the other measurements which show statistical data points within the same graph or table, the reviewer may extrapolate the SIG and NOSIG points for the other measurements as LOEC and NOEC using the R designation in the ASSIGNED ENDPT field. The reviewer may not extrapolate data from other tables or graphs. In studies where measurements are reported with statistical significance but the author does not report a NOEC and/or LOEC for any of the measurements, the reviewer does not code a NOEC and/or LOEC.

Example: The text contains the following statement: "The NOEC and LOEC for weight were found to be 10 ug/l and 20 ug/l, respectively." A table reports weight data as well as length and mortality data which is statistically analyzed.

| Conc (ug/l) | Length (cm) | Weight (mg) | Mortality (%) | * Sig at p<0.05 |
|-------------|-------------|-------------|---------------|-----------------|
| control | 21 | 30 | 0 | |
| 5 | 22 | 30 | 5 | |
| 10 | 20 | 24* | 5 | |
| 20 | 15* | 20* | 8 | |
| 30 | 13* | 18* | 25* | |

Code:

| Endpoint Assigned | Endpoint | Effect | Measurement | Conc | Signif | Level |
|-------------------|----------|--------|-------------|---------|--------|--------|
| P | NOEC | GRO | LGTH | 10 ug/l | NOSIG | p<0.05 |
| P | LOEC | GRO | LGTH | 20 ug/l | SIG | p<0.05 |
| R | NOEC | GRO | WGHT | 5 ug/l | NOSIG | p<0.05 |
| R | LOEC | GRO | WGHT | 10 ug/l | SIG | p<0.05 |
| R | NOEC | MOR | MORT | 20 ug/l | NOSIG | p<0.05 |
| R | LOEC | MOR | MORT | 30 ug/l | SIG | p<0.05 |

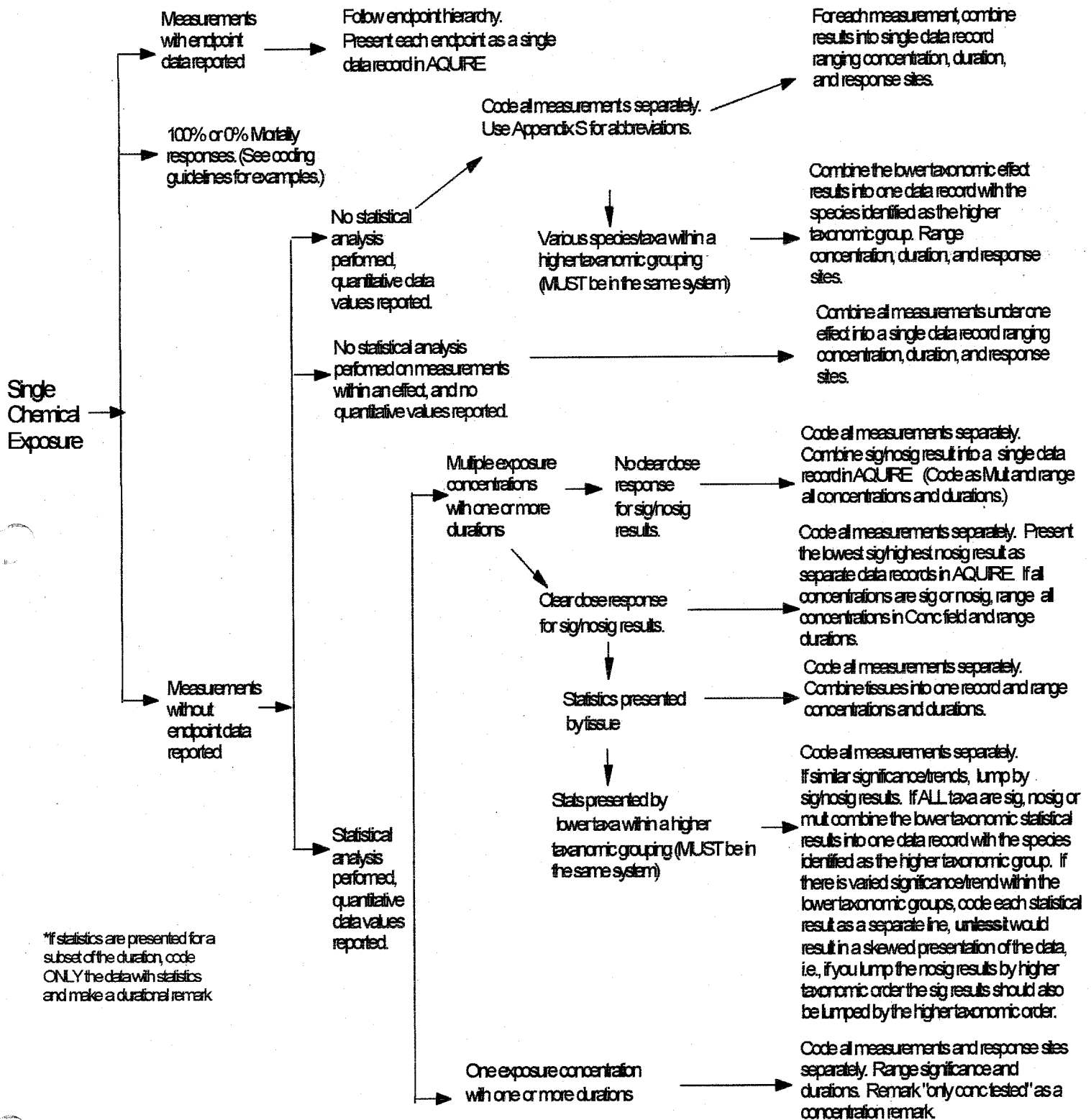
On occasion, authors will report LC50 information in the methods section of their publication, without reporting any accompanying test procedure information. These test results are coded if enough information is provided by the authors to verify that the value(s) were not published elsewhere, and that the study meets all five minimal criteria for acceptance.

If replicate tests resulting in a number of endpoints, (e.g. LC50s), are conducted, each LC50 must be reported on an independent line, even though the chemical, species, duration and effect are the same. Mean results are not coded if individual results are reported,

Example: Rep 1 - LC50 = 23 ug/L Code only Rep 1 and Rep 2.
 Rep 2 - LC50 = 25 ug/L
 Mean - LC50 = 24 ug/L

If a data set is evaluated using more than one statistical analysis all resulting endpoints are coded on separate lines (e.g. 2 LC50s for same data using probit and Spearman-Karber will be coded as two separate data lines; report statistical method in EE REMARK). Additionally, note "statistical comparison" in the OTHER EFCT field (see OTHER EFCT section for more information).

Figure 1. Hierarchy of coding tests in AQUIRE



*If statistics are presented for a subset of the duration, code ONLY the data with statistics and make a durational remark

ENDPOINT HIERARCHY

The following hierarchy defines the priority for including endpoint information in the AQUIRE database. The endpoints listed in category "A" are the highest priority, based on conformance with standard toxicity endpoints, and should be coded if reported in the publication. If the endpoints identified in subsequent categories (F) are also listed in the publication, these endpoints are not coded but are noted in the OTHER EFCT field. If there are no endpoints from category "A" in the publication, then endpoints from category "B", if available, are coded and so on.

Following the endpoint hierarchy, the next two sections define and describe the coding of trends and effects. Trend information is coded, when available for endpoints as well as effects. Regardless of whether endpoint data is available, any reported effect information is coded.

- A The endpoint is an LC50, LD50, ED50, LETC, EC50, IC50, NOEC, LOEC, MATC, LR50, ER50 or BCF (BCFD), or its definition, as reported by the author. For example, if the author does not actually state that the value is an LD50 but states that "concentration x is the dose estimated to be lethal to 50% of the test organisms" and refers to statistical methods to estimate 50% lethality, the reviewer should code this as an LD50 endpoint because the author *defines* the LD50. All individual endpoints are coded. If both BCF and BCFD (wet weight and dry weight, respectively) are presented, code only the BCFD.

The AQUIRE database recognizes and codes "companion endpoints"; for AQUIRE such endpoints are defined as statistically significant endpoints that neighbor an author-defined NOEC or LOEC.

When a publication reports a LOEC and NOEC for a non-monotonical response pattern (i.e., lower concentration significant and at least one higher concentration not significant); code the LOEC/NOEC reported by the authors, but note TREND as 'CHG' and code the SIG field as MULT to flag non-standard results.

In the AQUIRE database, the occurrence of no mortality (0%) or complete mortality (100%) is treated as an endpoint. The endpoints NR-LETH and NR-ZERO will always be coded for mortality effects of 100% mortality and 0% mortality, respectively. If for a laboratory test exposure the authors report "all fish died", code as NR-LETH and 100% mortality; however, for a field exposure, unless conducted in an enclosure of some type, it is difficult to assume that truly 100% of the fish are known to be dead, therefore the field exposure report of "all fish died" is coded POP, DEC, ABND, and EFCT% is not coded. The term "nil" is defined as "naught or nothing", therefore, when used by an author, it will be assumed to mean 0% mortality and coded as NR-ZERO.

The 100% mortality data point at the lowest concentration/ shortest duration is coded. Similarly, the 0% mortality data point at the highest concentration/ longest duration is coded. In contrast to other endpoints, the additional mortality effects are coded along with

the NR-LETH and NR-ZERO endpoint data. For example:

Mortality Table 1

| µg/L | 24 H | 48 H | 72 H | 96 H |
|------|-------------|------|------|-----------|
| 1 | 0 | 0 | 0 | 0 NR-ZERO |
| 2 | 5 | 17 | 30 | 35 |
| 3 | 25 | 40 | 65 | 90 |
| 4 | 100 NR-LETH | 100 | 100 | 100 |

A) LC50s reported in publication, code

LC50s as reported
 NR-LETH: 4 µg/L at 24 hr
 NR-ZERO: 1 µg/L at 96 hr

B) LC50s not reported in publication, code

NR-LETH: 4 µg/L at 24 hr
 NR-ZERO: 1 µg/L at 96 hr
 MOR: 2-3 µg/L at 24-96 hr

Mortality Table 2

| µg/L | 24 H | 48 H | 72 H | 96 H |
|------|------|-------------|-----------|------|
| 1 | 0 | 0 | 0 NR-ZERO | 11 |
| 2 | 20 | 25 | 38 | 72 |
| 3 | 45 | 60 | 67 | 90 |
| 4 | 90 | 100 NR-LETH | 100 | 100 |

A) LC50s reported in publication, code

LC50's as reported
 NR-LETH: 4 µg/L at 48 hr
 NR-ZERO: 1 µg/L at 72 hr

B) LC50s not reported in publication, code

NR-LETH: 4 µg/L at 48 hr
 NR-ZERO: 1 µg/L at 72 hr
 MOR: 1-4 µg/L at 24-96 hr EFCT%: 0-100

Mortality Table 3

| µg/L | 24 H | 48 H | 72 H | 96 H |
|------|---------------|------|------|---------------|
| 1 | 0 START RANGE | 0 | 7 | 13 |
| 2 | 0 | 28 | 45 | 60 |
| 3 | 38 | 44 | 67 | 100 |
| 4 | 38 | 60 | 100 | 100 END RANGE |

A) LC50s reported in publication, code

LC50's as reported

B) LC50s not reported in publication, code

MOR 1-4 µg/L at 24-96 hr EFCT% 0-100

Mortality Table 4

| µg/L | 24 H | 48 H | 72 H | 96 H |
|------|---------------|------|------|---------------|
| 1 | 0 START RANGE | 0 | 0 | 0 |
| 2 | 0 | 0 | 7 | 13 |
| 3 | 0 | 28 | 45 | 60 |
| 4 | 38 | 44 | 67 | 100 |
| 5 | 38 | 60 | 100 | 100 |
| 6 | 100 | 100 | 100 | 100 END RANGE |

A) LC50s reported in publication, code

LC50's as reported

B) LC50s not reported in publication, code

MOR 1-6 µg/l at 24-96 h, EFCT% 0-100

- B The endpoint is an author reported TLM, TL50, chronic value (ChV) or any terms with equivalent definitions that define endpoints such as those listed in ECOTOX Appendix T. The equivalent AQUIRE endpoint is coded in the ENDPOINT field.
- C The endpoint is LCxx, LDxx, ECxx, EDxx, ICxx, LRxx, ERxx (other than 50% value). The endpoint is coded only if the endpoints listed in A or B are not abstracted from the publication.
- D The endpoint is LT50, ET50. The endpoint is coded only if the endpoints listed in A and B and C are not abstracted from the publication.
- E The endpoint is LTxx, ETxx. The endpoint is coded only if the endpoints listed in A, B, C and D are not abstracted from the publication.
- F The endpoint is a delayed exposure effect (~xxx). The delayed effect endpoint is coded if no similar exposure endpoint above has been coded. A specific exception is gut clearance prior to tissue analysis; e.g., "after the exposure the organisms were placed in clean water for 10 hours to allow the organism to clear the stomach contents". This type of clearance is distinguished from depuration and is not coded as a delayed effect.

Assigned Endpoint (ASSIGNED ENDPOINT)

If the reviewer codes an endpoint which is not stated by the author in the paper, or interprets an author's endpoint definition to be equivalent to the AQUIRE endpoint definition, an "R" for reviewer-assigned endpoint is entered into the field. In all cases except for NR-ZERO and NR-LETH, "by definition", the endpoint the author reports or the description of the endpoint is coded in the EE REMARK data field. However, if the reviewer codes an endpoint as the author states it in the paper, a "P" is coded for paper-assigned endpoint.

Example 1. Author reports NOEC in paper but does not include companion endpoint - LOEC. If reviewer can determine LOEC from data, an "R" is coded for the LOEC (code "by definition" in the EE REMARK field) and a "P" is coded for the NOEC.

Example 2. Author uses TLM for 50% mortality endpoint. Reviewer can code the endpoint as LC50 and puts "R" for reviewer assigned endpoint (code TLM in the EE REMARK field).

Note: An "R" is always coded for the endpoints NR-ZERO and NR-LETH, however no further information is coded in the EE REMARK field.

Trend (TREND)

The observed or measured response trend as compared to the control is coded when reported or graphically displayed.

When assigning a trend to a record, it should reflect the measurement which may or may not reflect the effect. For example, when authors report a decrease in survival; the effect is reported as MOR, and the trend is associated with the measurement; i.e., decrease in survival.

Example: EFCT: MOR TREND: Dec MEASURE: SURV

The trend for BCF, LCxx, LTxx is coded as "inc", except for the effect SVC (shell valve closure) which is coded as "dec". The trend for ECxx, NOEC, LOEC, and MATC will be either "inc", "dec", "chg" or NR depending on the results of the test. In instances when a trend is non-monotonical code "chg". The trend is noted as a two or three letter code:

| | |
|--------------|--|
| <u>CODE:</u> | <u>TREND:</u> |
| INC | increase |
| DEC | decrease |
| NEF | no observed effect; e.g., when coding NR-ZERO the trend is NEF |
| CHG | no clear trend, results are variable (e.g. any combination of above trends listed) |
| NR | no trend reported <u>or</u> if no control response is reported then the trend is not able to be identified |

Example: When a clear response, or lack thereof, is observed within an effect, it is coded as either INC, DEC, or NEF. The measurement used to evaluate the effect is reported in the MEASURE field, for example:

EFCT: GRO TREND: INC MEASURE: LGTH

When measurements do not report quantifiable data (see EFFECT MEASUREMENT section), data are combined into one record. If these data report multiple trends, code CHG in the TREND field and report the individual trends in EE_REMARK field as in the following example:

Example: EFCT: HIS TREND: CHG MEASURE: GHIS
EE_REMARK: inc EDMA, vacuolization, dec epithelial lining

Effect (EFFECT)

For ECOTOX database purposes, a toxicological effect is the observation or measurement of a response resulting from the action of a chemical stressor (e.g., mortality). The ECOTOX database internally categorizes all observed effects under at least one of eleven major effect group codes (Accumulation, Behavior, Biochemical, Cellular, Growth/Development, Lethal, Physiological, Population Community, Reproduction, Ecosystem and multiple groups). ECOTOX Appendix R describes the major groups and associated effect definitions for each three letter code. The major effect groups are not used by reviewers; their purpose is to provide database users the capability to search on broad groups of effects without specifying each individual effect. See Scientific Outreach Support for additional user support information.

The reported effect is interpreted to conform to the AQUIRE defined effects. If the effect is on the list of AQUIRE effects, use the AQUIRE effect code (see ECOTOX Appendix S). If the author's

effect is not in Appendix S, but is similar to one already defined use the AQUIRE code which matches the definition and note the author's effect term in the EE REMARK field. If the author's effect appears to be a new effect code, discuss and forward to EPA Data Manager for approval.

Listed at the end of ECOTOX Appendix R there are two special effect code conventions used in AQUIRE. The first is NOC (No effect code) used only for ENDPOINTS reported by the author as multiple effects, e.g. "mortality and growth" and no specific effect code can be assigned. This code is used *only* when such effects cannot be separated into or reported as individual effects. The NOC code is rarely used and when used must be verified by one or more fully trained reviewers.

The second effect code convention is ~XXX to indicate that the result reported was observed after the exposure period ended and the organisms are observed in clean water, i.e., a delayed response. Within a publication, delayed response data is reported only if exposure period observations are not available for the same effect or endpoint. When delayed response data accompanies exposure period observations, the delayed response data is not coded but is recorded in OTHER EFFECT as "recovery".

NOTE: A specific exception is gut clearance prior to tissue analysis; e.g., "after the exposure the organisms were placed in clean water for 10 hours to allow the organism to clear the stomach contents". This type of clearance is distinguished from depuration and is not coded as a delayed effect.

Occasionally, effects describing a parasite-host relationship are coded in AQUIRE. For example, the effect on the host is typically coded as a PHY effect with the measurement code PRNF. The effect on the parasite is typically coded as a POP effect with the measurement code ABND.

EFFECT HIERARCHY

- A. If the author has defined an Endpoint for an effect, report the Endpoint as outlined in the preceding ENDPOINT HIERARCHY.
- B. When only effects are reported in the publication, no endpoints, code the concurrent effects (results reported concurrent with exposure to chemical) according to the abbreviations in ECOTOX Appendix S. Code NR (not reported) in the ENDPOINT field.
 - i. If statistics are presented in a clear dose response, code the lowest significant effect and the highest no sig levels and appropriate p-values.
 - ii. If statistics are presented and there is no clear dose response, code as a MULT and the appropriate value.
 - iii. If no statistics are used, or reported, combine the effect data by coding a range for concentration and duration. Report as NR in the SIG and LEVEL fields.
- C. When the only effects that are reported are those subsequent to exposure, report these as

delayed effects, noted with a ~ preceding the three-letter effect code, e.g. ~MOR. Follow the procedures outlined in Steps B i, ii, iii for reporting delayed effects.

Response Site (RESP SITE)

A response site code is used to identify specific organ and tissue effect sites for residue, biochemical and/or physiological effect measurements. For example, response sites are used for ACC, BIO, CEL, HIS, PHY, GRO, and MPH effects and associated endpoints. The two or three letter response site codes are listed in ECOTOX Appendix U. It is acceptable to code a response site when tissues or organisms are pooled together for a measurement.

If data for a number of tissues are presented along with statistical results report results for each tissue separately. If statistics are not presented, combine the results into one data record.

Combining Response Site

When the residue measured in one organ or tissue is further analyzed to indicate concentrations in cells or cellular fractions, a comment is placed in the REMARK field (e.g., SITE /subcellular fraction// or SITE /subcellular distribution//).

If the MT/ code is used, the individual tissues/organs are coded in the REMARK field (e.g., Site /LI, KI, GI//). If the response site does not have a response site code, write out the response site name and include a note with the coding sheet requesting a new code be added. When the response site is not reported, the field is coded as NR. If whole organism and multiple sites are listed, code "MT/" in tissue field and code WO and additional specific tissue codes in the REMARK field (e.g. SITE/WO, LI, GI, HE//).

Effect Measurement (MEASURE)

Generally, "measures" or "measurements" are variables used to aid in the interpretation of the degree of response to a toxicant by an organism. For example, measures of behavioral effects in ECOTOX include behavioral changes (BEH SWIM), changes in feeding activity (FDB FDNG), and stimulus avoidance (AVO STIM). ECOTOX Appendix S lists the measurements currently used for each of the effects in the ECOTOX database.

Each measurement that reports quantitative data (i.e. numeric values in the text, tables, or graphs), regardless of statistical analysis, receives a separate data line. However, measurements that are discussed in the text and do not report any numeric values may be combined into one record. If several measurements are combined, code the General measurement code in the MEASURE field and list the separate measurements in the EE REMARKS field.

Example 1: Length and weight reported in table statistically analyzed.

| | | | | | |
|------|---|---------|---------|---------|--------|
| CONC | 0 | 10 ug/l | 20 ug/l | 30 ug/l | 40ug/L |
|------|---|---------|---------|---------|--------|

| | | | | | |
|--------|---|-------|-------|-----|-----|
| Length | - | nosig | nosig | sig | sig |
| Weight | - | nosig | sig | sig | sig |

| | | | |
|-------|-----------|---------------|--------------------------------------|
| Code: | EFCT: GRO | MEASURE: LGTH | CONC: 20ug/L - nosig 30ug/L - sig |
|-------|-----------|---------------|--------------------------------------|

| | | | |
|--|-----------|---------------|--------------------------------------|
| | EFCT: GRO | MEASURE: WGHT | CONC: 10ug/L - nosig 20ug/L - sig |
|--|-----------|---------------|--------------------------------------|

Example 2: A decrease in length and weight reported on graph which is not statistically analyzed but values are given.

| | | | | | |
|-------------------|-----------|------------|---------------|--------|-----------------|
| CONC (from graph) | 0 | 10 ug/l | 20 ug/l | 30ug/L | |
| Code: | EFCT: GRO | TREND: DEC | MEASURE: LGTH | | CONC: 10-30ug/L |
| | EFCT: GRO | TREND: DEC | MEASURE: WDTN | | CONC: 10-30ug/L |

Example 3: Histology reported in text. At concentrations of 10 ug/l , 20 ug/l and 40 ug/l, Reports that liver had edema, swelling, and hypertrophy.

| | | | |
|-------|------------------|---------------|-----------------------------|
| Code: | EFCT: HIS | MEASURE: GHIS | EE remark: EDMA, SWEL, HYPT |
| | CONC: 10-40 ug/l | | |

More information on coding statistics is found in the statistics section of this SOP.

Many publications which report field data or laboratory microcosm studies present results for multiple species/taxonomic groups. The combining of results for species and taxonomic groups depends on whether statistics were applied to the data and whether a similar response is evident.

- If, within a higher taxonomic group (e.g., Algae), individual effects for several lower taxonomic groups are also presented (e.g., Bacillariophyta (diatoms), Chlorophycota (green algae), Pyrrophyphyta (dinoflagellates)) the data may be reported in a number of ways. Examples include:

- The measurements within each group are statistically analyzed and are similar overall, ie., INCreasing, DECcreasing or CHanGing. Combine the results and code as:

| | | | |
|----------------|----------------------|-------------|---------------|
| Species: Algae | ORG CHARAC: 3 orders | EFFECT: POP | MEASURE: ABND |
| TREND: INC | SIG: SIG | | |

- The measurements within each group are statistically analyzed and differ from each other. Code each result as a separate line:

| | | | | |
|--------------------------|-------------|---------------|------------|----------|
| Species: Bacillariophyta | EFFECT: POP | MEASURE: ABND | TREND: INC | SIG: SIG |
| Species: Chlorophycota | EFFECT: POP | MEASURE: ABND | TREND: DEC | SIG: SIG |

- If no statistical analysis has been reported, the results from the lower taxonomic groups can be combined into a single record representing the next highest representative taxonomic group.

Species: Algae ORG CHARAC: 3 orders EFFECT: POP MEASURE: ABND
TREND: CHG SIG: NR

EE_Comment (EE_REMARK)

This field contains additional endpoint and/or effect text, as described by the author. The types of information coded include:

Example 1: The endpoint terminology used by the author when an ECOTOX-defined endpoint was coded rather than the author's term. For example,

ENDPOINT: LC50 EE_REMARK: TLM or Median Period of Survival//

Example 2: If there are multiple measurements for the coded effect, all of the measurements are listed in the EE_REMARK field.

EFFECT:HIS MEASURE: GHIS EE_REMARK: EDMA, LESI, epithelial lining//

If there are no remarks pertaining to either the endpoint or the effect, the field is left blank. As much as possible, codes should be used in EE_REMARK, but use of text is appropriate to ensure an understanding of the test result.

Effect % (EFCT %)

The EFCT% field is used when the effect is reported as a percent change, e.g. percent of the total population or percent increase or decrease.

If the author reports the number dead (i.e., "5 of 20") do not recalculate as a percent.

Example 1: "80% mortality" EFCT: MOR TREND: INC EFCT %: 80
MEASURE: MORT

Example 2: "25% survival" EFCT: MOR TREND: DEC EFCT%: 25
MEASURE: SURV

Example 3: "5 of 20 died" EFCT: MOR TREND: INC EFCT%: NR
MEASURE: MORT

Example 4: "45% inc ATPase activity" EFCT: ENZ TREND: INC
EFCT%: 45 Measure: ATPA

If the percent effect is coded from a graph, code the percent values using a qualifier, ie. <, >, or ~,

using only the graphical intervals reported on the graph. Place a slash in the field and code EFCT%/ from graph// in the REMARK field. If the percent effect is graphed and is not clear enough to extrapolate, code "graphed" in EFCT % field. If the effect percent is not reported, the field is coded as NR.

If the percent effect is presented as "xx% of the control", place a "/" in the EFCT % field and code: EFCT %/xx% of control// in the REMARK field.

Example: GRO WGHT Dec 20-30% of the control

EFCT: GRO MEASURE: WGHT EFCT%: / TREND: DEC Remarks: Efct%/20-30% of control//

Combining Effect Percent

When data for an effect are combined because a statistical analysis was not applied and/or a clear dose response was not observed, and several percent effect values are presented, there are two different ways to report data.

If the author reports the effect measurement on a single parameter, the effect percent is reported as a range.

Example 1: 30-75% mortality EFCT: MOR MEASURE: MORT TREND: INC
EFCT%: 30-75

Example 2: 20-30% dec O₂ consumption EFCT: PHY MEASURE: OXYG TREND: DEC
EFCT%: 20-30

Statistical Significance (SIG)

The statistical significance field is coded when the author has presented statistical analysis as compared to the controls in the test result.

The valid codes for this field are:

| <u>CODE</u> | <u>DEFINITION</u> |
|-------------|---|
| SIG | Concentration(s) identified as significant (code lowest concentration in series of significant treatments) |
| ASIG | All toxicant concentrations significant |
| NOSIG | Concentrations identified as not significant (code highest concentration in series of non-significant treatments) |
| ANOSIG | All toxicant concentrations not significant |
| MULT | Combination of sig and nosig results; pattern of significance is non-monotonical, or results combined due coding practices. |
| NA | Not applicable (use for LC50, EC50, BCF, MATC, NR-LETH, AND NR-ZERO) |
| NR | Not reported |

If statistics are presented in the publication, unless the authors state otherwise, assume that the

exposure treatments were compared to the controls.

When statistical comparisons are presented for multiple controls (e.g., statistics in relation to a standard control and statistics in relation to a solvent control), code **M** in the CONTROL TYPE field and note the appropriate control type used for the coded statistics in EE REMARK (e.g., stats based on solvent control//) The data related to the solvent control is coded preferentially over the standard control data and should be noted as such in the EE REMARK field.

Data is separated into individual records if statistics are based on concentrations and a clear dose response is shown. However, responses reported over time are exceptions to this rule. For example:

- 1) If only one concentration is presented and the response differs over time, code one record, ranging the durations.

Example 1:

| Conc. | 24h | 48h | 72h | 96h |
|--------|-------|-------|-----|-----|
| 1 ug/l | nosig | nosig | sig | sig |

Code: CONC: 1 ug/l EXP TIME: 24-96 h SIGNIF: MULT

- 2) If multiple concentrations are presented and the results are non-monotonic across concentrations and time, code one record combining all concentrations and all durations.

Example 2:

| Conc. | 24h | 48h | 72h | 96h |
|--------|-------|-------|-------|-----|
| 1 ug/l | nosig | nosig | nosig | sig |
| 2 ug/l | nosig | nosig | sig | sig |
| 3 ug/l | nosig | sig | sig | sig |

Code: CONC: 1-3 ug/l EXP TIME: 24-96 h SIGNIF: MULT

- 3) If multiple concentrations are presented and the results are monotonic across concentrations and time, code two records, a NOSIG record combining all durations and a SIG record combining all durations.

Example 3:

| Conc. | 24h | 48h | 72h | 96h |
|--------|-------|-------|-------|-------|
| 1 ug/l | nosig | nosig | nosig | nosig |
| 2 ug/l | sig | sig | sig | sig |
| 3 ug/l | sig | sig | sig | sig |

Code: CONC: 1 ug/l EXP TIME: 24-96 h SIGNIF: NOSIG
Code: CONC: 2 ug/l EXP TIME: 24-96 h SIGNIF: SIG

The SIG field is coded as "NA" for records having an endpoint of MATC, LCxx, ECxx, LTxx, BCF, ETxx, ICxx, LDxx, LETC, BCFD, NR-LETH, and NR-ZERO. For NOEC, LOEC and effects without endpoints, code significance as author reports, or NR.

When a publication reports a LOEC and NOEC for a non-monotonical response pattern (i.e., lower concentration significant and at least one higher concentration not significant); code the

LOEC/NOEC reported by the authors, but note TREND as 'CHG' and code SIG as 'MULT' to flag non-standard results.

The reviewer interprets hypotheses tests to determine a dose response endpoint. A significant clear dose result is coded as SIG; no significant dose result is coded as NOSIG. Only the highest NOSIG and the lowest SIG concentration is reported; unless all concentrations are SIG or all concentrations are NOSIG. In this instance, code all the concentrations as a range as ASIG (all significant) or ANOSIG (all not significant) respectively. If the significance level is reported, it is coded in the LEVEL field described below.

In cases where the author reports only a SIG or NOSIG, code the companion data point. For example, if a stat sig "growth" is reported in the text and in the table sig is noted the reviewer should pick the nosig level and report this also.

If the author states that there is a statistically significant increase or decrease in an observed effect, whether or not they report the statistical method used, but does not report a significance level, code SIG or NOSIG and NR in LEVEL field.

If a table has a footnote defining * values as significant as $p < xx$, it is acceptable for the reviewer to assume that data points without an asterisk are not significant.

If the author states there is a significant increase or decrease in an observed effect but does not say it is "statistically significant," code NR in SIG field.

- When the highest concentration and all lower concentrations tested show no significant response, code ANOSIG and range all concentrations in CONC field.

Example:

| | | |
|---------|-------|--------|
| 10 ug/L | NOSIG | P<0.05 |
| 20 ug/L | NOSIG | P<0.05 |
| 30 ug/L | NOSIG | P<0.05 |

Code: CONC: 10-30 ug/L SIG: ANOSIG LEVEL: P<0.05

- When the lowest concentration and all higher concentrations tested show a significant response, code ASIG and range all concentrations in CONC field.

Example:

| | | |
|---------|-----|--------|
| 10 ug/L | SIG | P<0.05 |
| 20 ug/L | SIG | P<0.05 |
| 30 ug/L | SIG | P<0.05 |

Code: CONC: 10-30 ug/L SIG: ASIG LEVEL: P<0.05

- If only one concentration is tested and statistics are performed, code SIG or NOSIG in stats and "only conc tested" as a CONC remark.

Example:

| | | |
|---------|-----|--------|
| 10 ug/L | SIG | P<0.05 |
|---------|-----|--------|

Code: CONC: 10ug/L SIG P<0.05 REMARK: Conc/only conc tested//

Combining of Statistics

If a measurement has no clear dose response as interpreted by the reviewer when statistics are

reported, it is coded as multiple significance (MULT).

Example 1: Five concentrations are tested and the two highest and two lowest show significance but the middle concentration does not, code MULT.
Growth table with length affected by copper.

| | | | | | | | |
|--------|-------------------------|--------|--------|--------------|--------|---------------|--------|
| Conc | Control | 1 ug/L | 2 ug/L | 3 ug/L | 4 ug/L | 5ug/L | |
| Length | 20 cm | 35cm | 36cm | 21cm | 15cm | 14cm | |
| | | sig | sig | nosig | sig | sig | P<0.05 |
| Code: | EFCT: GRO MEASURE: LGTH | | | SIGNIF: MULT | | LEVEL: p<0.05 | |
| | CONC: 1-5 ug/L | | | | | | |

Example 2: Change in calcium concentration in the blood over three sample times shows significance at 24 hours, no significance at 48 hours, and significance at 96 hours. Code SIG field as MULT.

| | | | | |
|-----------|---------------|-------------------|--------------|---------------|
| [Calcium] | 24 hours | 48 hours | 96 hours | |
| Control | | | | |
| 10 ug/L | sig | nosig | sig | P<0.05 |
| Code: | EFCT: BCM | MEASURE: CACO | SIGNIF: MULT | LEVEL: p<0.05 |
| | CONC: 10 ug/L | DURATION: 24-96 H | | |

Example 3: A publication reports a LOEC and NOEC for a non-monotonical response pattern (i.e., lower concentration significant and at least one higher concentration not significant); code the LOEC/NOEC reported by the authors, note TREND as 'CHG', and code SIG as 'MULT' to flag non-standard results.

| | | | | | | |
|------------------|--------------|------------|---------------|---------------|---------|---------|
| Mortality | Control | 10 ug/L | 20 ug/L | 30 ug/L | 40 ug/L | 50 ug/L |
| @ 24 hours | | nosig | nosig | sig | nosig | sig |
| Author endpoints | | | NOEC | LOEC | | |
| Code: | ENDPT: NOEC | EFCT: MOR | MEASURE: MORT | CONC: 20 ug/L | | |
| | SIGNIF: MULT | TREND: CHG | | | | |
| | ENDPT: LOEC | EFCT: MOR | MEASURE: MORT | CONC: 30 ug/L | | |
| | SIGNIF: MULT | TREND: CHG | | | | |

Note: The MULT code is not used to represent a combination of data which has been statistically analyzed with data that has not been analyzed. Code the statistical data over the non-statistically analyzed data. For example, in an instance where the exposure duration is 5 days, and the statistical results presented are for 4 days; code the statistical results, note the duration as 4 days., place a slash in the duration field, and enter the exposure duration (5 days) in the Remark field.

Significance Level (LEVEL)

The level of significance (e.g. test statistic) is coded when the author has reported statistical analysis in the test result. The terminology for significance level may be presented as: p =; p- or alpha value; ²; for t-test; % level. The terminology are equivalent and are generally in the range of p = 0.10 to p = 0.001.

The LEVEL field is coded as "NA" for records having an endpoint of MATC, LCxx, ECxx, LTxx, BCF,

ETxx, ICxx, LDxx, LETC, BCFD, NR-LETH, and NR-ZERO. However, when the confidence level is other than 95%, the level is coded as reported.

Combining of Level

When a range of concentrations is coded, and there are multiple levels of significance reported, range the values.

Example: At all concentrations (10-50 ug/L) growth was significantly affected. At 10 ug/L the p value was $p < 0.05$, at 50 ug/L the p value was $p < 0.001$.

CONC: 10-50 SIG: SIG LEVEL: $P < 0.05 - 0.001$

Other Effects (OTHER EFCT)

Comments regarding other toxicity tests or effects reported in the publication that do not meet AQUIRE minimum data requirements are coded in this field. A keyword list (see ECOTOX Appendix V) for common terms is used as a guideline to assist the reviewer. The effect or endpoint codes are used when appropriate. The reviewer should maintain a list of new keywords and periodically submit this list to the EPA Database Manager. Commas separate each distinct term and the text ends with a double slash (//).

OTHER EFCT: uptake, elimination//

OTHER EFCT: toxicity symptoms, diet study//

OTHER EFCT: mixture, effluent//

If other chemicals are tested as a mixture with the test chemical, the keyword "mixture" is coded in the OTHER EFCT field.

When water chemistry effects (temperature, salinity, pH) are tested in conjunction with chemical toxicity, a Remark is coded in OTHER EFCT to reflect this type of interaction.

OTHER EFCT: salinity efcts//

Test Result Examples

1. If the author has defined an ENDPOINT and/or has reported a 0% and/or 100% mortality response, report the endpoint/mortality as outlined in the Endpoint Hierarchy. Select the appropriate effect as described below.

ENDPOINT REPORTED (NR-ZERO):

ENDPOINT: NR-ZERO

MEASURE: MORT

TREND: NEF

EFCT%: 0

EFFECT: MOR

SIG: NA

LEVEL: NA

If applicable, statistical results should appear in the SIG field, the level of significance should be reported in the LEVEL field, the percent effect should be presented in the EFCT% field, and the trend

should be reported in the TREND field.

ENDPOINT REPORTED (LOEC):

| | | |
|----------------|---------------|------------------------|
| ENDPOINT: LOEC | MEASURE: LGTH | |
| TREND: DEC | EFCT%: 20 | |
| EFFECT: GRO | SIG: SIG | LEVEL: $\alpha < 0.05$ |

Note: For NOEC endpoints, NOSIG is coded in the SIG field. For LOEC endpoints, SIG is coded in the SIG field.

2. If the author-reported effect is a clear dose response result using statistical analysis, and the author does not identify an endpoint, select the appropriate effect from ECOTOX Appendix S.

Clear dose response data where a statistically significant effect was observed, are represented by two data records. One data record represents the lowest concentration at which a statistically significant effect occurred. "SIG" is coded in the SIG field, the observed trend is coded in the TREND field, the percent effect is coded in the EFCT% field, and the level of significance is reported in the LEVEL field. The observed measurement is coded in the MEASURE field. Remarks on the effect are made in the EE_REMARK field.

CLEAR DOSE RESPONSE:

| | | |
|---------------|-------------|------------------------|
| ENDPOINT: NR | SIGNIF: SIG | TREND: DEC |
| EFFECT: GRO | EFCT%: 20 | LEVEL: $\alpha < 0.05$ |
| MEASURE: LGTH | | |

The second data record represents the highest concentration at which no effect occurred. NOSIG is coded in the SIG field. If a percent effect is reported it is presented in the EFCT% field.

If the concentration identified as SIG is the lowest concentration reported or the concentration identified as NOSIG is the highest concentration reported, report the range of concentrations and the appropriate code (ASIG and ANOSIG) in the SIG field.

If only one concentration is tested, code the SIG field appropriately and note "only conc tested" as the concentration (CONC) remark in the REMARK field.

3. If the author reported effect shows unclear dose response results, using statistical analysis, select the appropriate effect from ECOTOX Appendix S.

When data have been statistically analyzed, and the results presented have significant effects in an unclear dose response pattern (e.g., significant effects at the high and low concentrations, and not significant at the middle concentration), "MULT" is coded in the SIG field to signify multiple significance. The level is coded with a full range of p-values (e.g. $p < 0.05$ -0.001).

UNCLEAR DOSE RESPONSE:

| | | |
|--------------|---------------|---------------------|
| ENDPOINT: NR | MEASURE: ACHE | |
| TREND: CHG | | |
| EFFECT: ENZ | SIG: MULT | LEVEL: P<0.05-0.001 |

4. If the author reports a descriptive or qualitative effect without statistical analysis, select the most appropriate effect from ECOTOX Appendix S. One record is coded with a full range of exposure concentration and time. The appropriate trend is coded in the TREND field. The percent effect over the concentration tested is reported in the EFCT% field. NR is coded in SIG and LEVEL fields.

NO STATISTICAL ANALYSIS:

| | | |
|--------------|---------------|--------------------------------------|
| ENDPOINT: NR | MEASURE: GHIS | EE REMARK: LESI, ATRS, DEGN, EDMA |
| TREND: INC | EFCT%: NR | |
| EFFECT: HIS | SIG: NR | LEVEL: NR |

6. Concentration Parameters

Concentration Type (CONC TYPE)

The three forms of toxicants evaluated in AQUIRE are organic compounds, metals and inorganic non-metals. Each form can be identified as a concentration type code using the single letter abbreviation.

Organic compounds are defined by the pesticidal terms, formulation (F) and active ingredient (A). Publications that do not specify the compound by the definition criteria for active ingredients are by default coded in the formulation (F) category.

Metals are defined by the concentration types, total (T), dissolved (D), and labile/free (L); while ammonia or hydrogen sulfide compounds may have total concentrations (T) and/or un-ionized (U) concentrations. Organometals are coded as total (T) concentrations.

If two representative concentrations of a metal or inorganic non-metal are reported in the reference, both concentrations are included in the same AQUIRE record; i.e, both total and un-ionized concentrations are reported in the concentration field. If the author reports the ammonia concentrations as based on NH₄-N or NH₃-N, code CONC TYPE as "T" and "U", respectively in the same record and code the specific ion information in the ION fields.

For publications where all three metal types, T, D and L, are reported code T and D as one entry and the L concentration is coded as a separate line. (At some future point when new software is developed, all three concentration types will be associated with one record).

Concentration Type is also linked to the Chemical Analysis Method (METHOD) field. (see discussion below on Active Ingredient).

Concentration Types and Definitions

Organic:

FORMULATION (F): Way in which basic pesticide (toxicant) is prepared for practical use (Ware, 1978). Generally reserved for commercial preparation prior to actual use and does not include the final dilution (Insect-Pest Management and Control, 1971) (e.g.; Baythroid, 2,4-D). Also included in this category are organic compounds with no pesticidal activity (e.g.; PCB, dioxin).

ACTIVE INGREDIENT (A): Chemical substance in a product that is responsible for the pesticidal (toxic) effect (Ware, 1978). Reported as "A" when the author refers to the concentration as active ingredient, active principle or various grades of reagents (ie., Analytical, Reagent or Technical). When coding, a value in the publication may be reported as "Al kg/ha" or "kg Al/ha"; in AQUIRE this type of value is reported as 'A =' for CONC TYPE, with units as kg/ha. For example, 100 kg Al/ha is reported as A = 100 kg/ha.

Note: Information reported in the PURITY field does not necessarily determine whether concentration is A or F. In addition to the description above, using "A" as the concentration type occurs in situations such as the following:

- 1) Author states concentration of pesticide as "Al".
- 2) Author states %Al (PURITY) and reports measured concentration.
- 3) Author states measured concentration of a pesticide.

Metal/Organometals:

TOTAL (T): The concentration of metals determined on an unfiltered sample after vigorous digestion, or the sum of the concentrations of metals in both dissolved and suspended fractions (APHA et.al. 1992). Heavy metals and single elements (e.g. Na, Cl, Br) are coded as T.

DISSOLVED (D): Those constituents of an unacidified sample that pass through a 0.45 um membrane filter (e.g. soluble metal) (APHA et.al. 1992).

LABILE (L): The labile or free ion metal concentration determined by various analytical methods. When coding, the specific labile forms or complexes are not differentiated.

Inorganic non-metals:

Concentrations of ammonia and hydrogen sulfide are reported in the literature in either the total or unionized form. Code the form as specified by the author. Ammonia may be reported as a variety of different forms, eg., NH_3 , NH_4^+ , $\text{NH}_3\text{-N}$, NH_4OH , or NH_4Cl . (US EPA 1979) The author must state whether the form is Total or Unionized; T is the default for ammonia and hydrogen sulfide papers that do not state whether total or unionized concentrations are reported.

TOTAL (T): The dissociated, charged form of nitrogen or hydrogen related chemicals. This can take on numerous forms, e.g.; ammonium (NH_4^+), nitrite (NO_2^-), etc. (Rand and Petrocelli, 1985). T is the default for publications that do not state whether Total or Unionized concentrations are reported.

UN-IONIZED (U): The undissociated, uncharged form of ammonia or hydrogen sulfide. The ammonia molecule, NH_3 , is the unionized form. (In aqueous solution, ammonia assumes an equilibrium between NH_3 and NH_4^+ .) The NH_3 is the toxic entity of the ammonia compound (Rand and Petrocelli, 1985).

Ionic Fraction (ION)

For ionizing substances (e.g., metals, ammonia), report the dose as the ion if the concentration presented by the authors is reported as based on the ionic form of the compound (eg., organotin as Sn). Code the appropriate ionic symbol in the ION field (see ECOTOX Appendix O for Ion codes). If concentration is based on the total compound, code 'NR' in this field. For non-ionizing substances, code 'NR' in this field.

If the test chemical for a metal is reported as the elemental metal (i.e. mercury) code the ion (Hg) in the ION field.

Effect Concentration (CONC)

Report the effect concentration in the same units used by the author. Do not convert any units. (See ECOTOX Appendix N for a list of concentration units). The confidence interval, fiducial limits, or range is recorded when available. The water concentration is coded in this field, except for diet studies, where the concentration in the food is coded. If a test is run with two sources of chemical, such as diet and water, code the concentration of the diet in the CONC field, the EXP TYPE field as D and code CONC/water conc rpt// in the REMARK field.

When coding numbers do not use commas. They can be mistaken for decimal points or numbers.

Code the mean and range of a stated concentration unless there are multiple test results being combined into one test record. In this case the lowest minimum concentration and the highest maximum concentration will be used.

Example 1: Endpoint reported as LC50 = 100 (50-150) ug/L
Code: ENDPOINT: LC50 CONC: 100 (50-150) ug/L

Example 2: Effect (reported at 1 concentration tested with replicates)
Rep1 = 10 (9-13) ug/L
Rep2 = 11 (8-13) ug/L

Code: The lowest minimum value and the highest maximum value are coded.
Conc: 8-13 ug/L

Example 3: Multiple concentrations reported with no statistics. Combine concentrations into one record coding the lowest minimum value and highest maximum value.

Nominal Conc. Measured Conc.
1 ug/L 1.5 (1-2) ug/L
2 ug/L 2.3 (2-3) ug/L
3 ug/L 3.1 (2.5-3.3) ug/L

Code: Conc: 1 -3.3 ug/L

Occasionally an author will report a concentration as a % or fraction of an LC50 value; e.g., either the sublethal concentration used was "10% of the 96-h LC50" or "1/10, 1/15 and 1/20 of the LC50". Such concentrations may be recalculated and used as the effect concentration if the original LC50 concentration is provided in the publication. Flag the recalculation in the paper so that the calculation may be QA'd and document the recalculation in the margin or on a blank page of the publication. Put a slash next to the concentration value and note in the REMARK field: CONC/Recalculated//.

When concentrations are taken from a graph, put a slash next to the concentration value and note in the REMARK field: CONC/from graph//.

All reported concentrations are coded and identified as to whether the concentration is based on the active ingredient or formulation, or as the total, un-ionized or dissolved concentration, are identified (see CONC TYPE).

In certain cases, the AQUIRE concentration is routinely reported as some form of the test chemical. For metal salts, the concentration is generally expressed as ug ion/L (e.g., Hg⁺). Be sure to code the ION field with appropriate ion.

An exponential number greater than +8 or smaller than -7 (e.g., 1 x 10⁸; often reported as 10⁸) is coded as E+n or E-n (e.g., 1 E+8). The concentration field is 10 characters long, therefore numbers less than or equal to +8 or -7 can be written out, eg. 10⁶ is reported as 1,000,000.

When the concentration is reported as the metal (e.g., Sn), but the chemical tested is identified as an organometallic (tributyltin chloride (C₁₂H₂₇ClSn)): Record the full name of the chemical tested in the TEST field, enter "T" in the CONC TYPE, report the concentration in the CONC field, and identify in the ion that the concentration is based on the metal component and report in the ION field.

If a chemical concentration is reported in the control water, 'contaminated controls' should be noted in the EXP DESIGN field. The concentration of chemical in the controls is not coded.

If the chemical used is an aged solution and the author is comparing the data to varying aged solutions (e.g. 2 d aged solution, 4 d aged solution and 6 d aged solution), all data are coded, "X d aged solution" is coded in the EXP DESIGN field, and "Aged Solution Efcts" is coded in the OTHER EFCT field.

For field data, the water concentration may be reported as NR, if the application rate is reported (see AP RATE field). However, the concentration type (F,A,T,D,L,U) must still be coded in this field along with NR.

Bioconcentration Value (BCF)

The bioconcentration factor (BCF) is a unitless value describing the degree to which a chemical can be concentrated in the tissues of an organism in the aquatic environment. At apparent equilibrium during the uptake phase of a bioconcentration test, the BCF is the concentration of a chemical in one or more tissues of the aquatic organism divided by the average exposure concentration in the water. The unitless number is calculated by dividing the concentration of the exposure chemical found in the tissue by the concentration of the chemical found in the exposure water,

$$BCF = \frac{\text{g/kg chemical in organism tissue}}{\text{g/L chemical in H}_2\text{O}}$$

or it is calculated from a ratio of rate constants, if at steady state,

$$BCF = \frac{K1 \text{ (uptake)}}{K2 \text{ (elimination)}}$$

A bioconcentration endpoint is coded as either wet (or unknown) or as dry weight (BCF and BCFD, respectively). An accumulation (ACC) effect, measurement of RSDE, and the associated BCF value is coded in the BCF field. If the author does not calculate a BCF, the test is recorded as an ACC effect, measurement of RSDE, NR in the ENDPOINT field, and NR in the BCF field.

If a BCF is reported for the parent compound and for a metabolite, record the parent compound BCF and note /metabolite BCF// in OTHER EFCT.

If the BCF is at steady state or equilibrium, it is noted using the term "steady state" in the EE_REMARK field.

If the BCF is normalized for lipid, "lipid normalized" and the % lipid, if available, are reported in the EE_REMARK field.

EE_REMARK: Steady State, lipid normalized 5% lipid//

If an author reports more than one type of BCF, ie. lipid normalized, regular, or radioactive equivalents, for the same data point; code lipid normalized over regular and regular over radioactive equivalents. The secondary analysis endpoint is reported in OTHER EFCT.

OTHER EFCT: radioactive equivalent BCF//

For papers that report BCFs and provide Lethal Body Burden information, note "Lethal Body Burden" in OTHER EFCT. However, in a publication reporting only residue data as lethal body burdens code the effect as ACC, the measurement as RSDE, and report "Lethal Body Burden" in EE_REMARK.

If an author indirectly measures the uptake of a chemical in an organism by measuring the loss of the chemical from the test media, the data is not coded. However, if the chemical is measured in the excrement (urine or feces) it is coded as RSDE in urine (UR) or feces (FC).

NOTE: BCFs of less than 1 and negative BCF values are suspect and should be looked at by the EPA data base coordinator. Reviewers will code the data and send the paper on to the EPA data base coordinator for review and approval. The BCF's less than 1 are coded as reported by the author and negative BCF values are coded as <1.

Exposure Type (EXP TYP)

Exposures must either be aqueous, through the diet, or by injection. Specific exposure types are coded. For example, if an injection exposure type is reported, code the specific route of injection (such as IM for intramuscular). *In vitro* toxicity test results are not coded in the AQUIRE database. If an exposure type is not clearly defined or is not reported, an NR exposure type is coded. Exposure Type codes are listed in ECOTOX Appendix J.

Application Frequency (APP FREQ)

Report the frequency of the dose application in the APPLICATION FREQUENCY data field. Refer to Appendix K for application frequency codes and units. If the exposure type is flow-through or

continuous flow in lab studies, code "NR" as the frequency and "CON" as the unit in the APP FREQUENCY field. Examples of continual exposures for field data include artificial stream experimental systems and in situ exposures. If the exposure type for a lab study is static code "1" as the frequency and "X" as the unit in the APP FREQUENCY field. If an application frequency is not reported, record NR.

Note: The "X" in an application frequency unit represents the number that is written prior to the code.

Examples:

The author reports that the water was renewed every 48 hours. Code:
APP FREQ: 48 E X H

The author reports that the chemical was applied 3 times a month. Code:
APP FREQ: 3 X per MO

The author reports that the chemical was applied as a pulse dose of 3 hours every day. Code:
APP FREQ: 3 H per D

Chemical Analysis Method (MU)

This parameter identifies whether quantitative analyses of the toxicant concentration in the test water was conducted and whether measured concentrations were used to report the results. This field represents/defines the concentration which was used in reporting the endpoint or effect; publications may report **Measured** and **Unmeasured** concentrations for one test scenario, use the code which represents whether the specific effect/endpoint concentration was measured or unmeasured. If both measured and unmeasured concentrations for the specific effect/endpoint are reported, record only the measured concentrations. When chemical measurements are conducted on stock solutions, but nominal concentrations are reported for effects or endpoints, code as **Unmeasured**. When chemical measurements are conducted periodically throughout the exposure but the reported measurements are not correlated with the effects, code as **Unmeasured**. When chemical measurements are conducted periodically throughout the exposure and the effects are coordinated with the measurements, code as **Measured**. For non-English publications, code as **Not Reported** unless explicitly stated to be measured or unmeasured concentrations.

Even if measured values are reported by the author to have deteriorated by the end of the exposure, the **Measured** code should still be used. It is acceptable to assume that if the author used measured concentrations in residue analysis, that these measured values were carried over to calculate BCF's. (See ECOTOX Appendix P for codes)

7. Test Duration Parameters

Exposure Duration (EXP TIME)

Exposure duration is coded using the units reported in the publication. If exposure duration is not reported, the publication is rejected (unless it is an abstract or is a non-English publication). Time information may be extracted from a figure.

For a fluctuating or intermittent dosing (P) experiment, the total test time is recorded in the EXP TIME field with the exposure times and intervals between dosages reported in the APPLICATION FREQUENCY field.

Example: The author reports that the organisms were dosed with three pulses that were 45 minutes each in a 24 hour period. The test was run for 48 hours.

EXP TIME:48 HAPP FREQ: 3 UNIT: X, 45 MI for 24 H

When an exposure duration is not directly linked to a response, the duration is reported as the full range of time, e.g. "during a 10 week period" is coded as "0-10 wk" or if the response is for a portion of the exposure duration, ie., from day 2 through 10 wk, then code as 2-70 d.

For delayed effects, report the duration of exposure to the toxicant only. The observation time is not recorded. (See ECOTOX Appendix I for valid duration units)

For injection, diet, topical and environmental exposures where the actual exposure is dependent on biological and environmental conditions, the exposure time is recorded as equivalent to the study time. This assumption is made to ensure consistency in data representation; it is not necessarily a true reflection of the exposure time.

For generational studies, report results only for lifestages that are directly exposed to the toxicant.

Example 1: Parent exposed – Offspring in clean water.
Code effects on parents and code "generational study" in OTHER EFCT.

Example 2: Parents exposed – Offspring exposed
Code the effects on both parents and offspring. Code the initial lifestage of organisms tested in the ORGANISM CHARACTERISTICS field and code the lifestage of the organism being measured in the EE REMARK field.

When coding the endpoints LTxx or ETxx, which are based on the time it takes to get a XX% response, code the associated effect response time and not the total exposure time in the duration field.

Example 1: A test with algae is run for 4 hours, but it takes 41 minutes for the "T1/2" duration which is the time of PSII half-inactivation. The author reported T1/2 endpoint is similar to an ET50 endpoint, therefore a reviewer assigned ET50 is coded.

Code: Assigned Endpoint: R Endpoint: ET50 Effect: PHY Measurement: PSII Duration: 41 mi

Example 2: A 96 hour test is run with fathead minnows and reports mortality. The author reports the LT50 value at 10 ug/l, 20 ug/l and 30 ug/l as 3.5 days, 2 day and 1 day, respectively.

Code:
Assigned Endpoint: P Endpoint: LT50 Effect: MOR Measurement: MORT Duration: 3.5 d
Concentration: 10 ug/l

Assigned Endpoint: P Endpoint: LT50 Effect: MOR Measurement: MORT Duration: 2 d Concentration: 20 ug/l

Assigned Endpoint: P Endpoint: LT50 Effect: MOR Measurement: MORT Duration: 1 d
Concentration: 30 ug/l

8. Water Chemistry Parameters

The following water chemistry parameters are included in AQUIRE, and are coded in appropriate fields. These measured values pertain either to the test water chemistry or the dilution or culture water chemistry values. In the absence of test water chemistry parameters, it is acceptable to report the culture, holding tank, acclimation, control or dilution water, or pretest conditions denoted by an asterisk (*). Water chemistry parameters measured prior to or after the exposure period are coded only if test water chemistries are not reported in the publication. If the author reports the test conditions as "similar" to other methods in the paper, code test conditions as "NR".

When water chemistries differ between samples (e.g., test chamber or water body), and results are obtained from only some of the samples, water chemistries should be reported for only those samples actually tested.

If the parameter unit is any unit other than the standard unit code (see Table 2) the unit is coded with the value. When the author refers to the water chemistry values as approximate a "~" is coded in front of the value. Graphed data are coded as a range or as "less than" or "greater than" values and the term "graphed" is noted as a REMARK, e.g. temp/graphed//.

Water chemistry values should be coded as reported by the author. If the author uses the standardized units of the AQUIRE, the units do not need to be recorded. (See ECOTOX Appendix W for additional water chemistry units)

Table 2. Specific Parameters

| Field Name | Standard Unit | Definition | Comment |
|-------------|--------------------------|-------------|---|
| <u>TEMP</u> | C | Temperature | When temperatures are reported for incubation chambers or water baths, these temperatures are acceptable for reporting as test temperatures. Do not code temperatures noted as "room temperature". |
| <u>HARD</u> | mg / L CaCO ₃ | Hardness | If the author only reports the terms "hard" or "soft", these terms are recorded. If the author reports a hardness value but does not identify a unit and/or refers to the value as "total", standard units are assumed and the value coded. |

| | | | |
|--------------|--------------------------|------------------|--|
| <u>ALK</u> | mg / L CaCO ₃ | Alkalinity | If the author reports an alkalinity value but does not identify a unit and/or refers to the value as "total", standard units are assumed and the value coded. |
| <u>DO</u> | mg / L | Dissolved Oxygen | A "SAT" code is used for 100% saturation |
| <u>pH</u> | -- | pH | pH range is between 1 and 14 |
| <u>SALIN</u> | ppt | Salinity | |
| <u>COND</u> | umhos / cm | Conductivity | |
| <u>ORG C</u> | mg / L Carbon | Organic Carbon | Organic carbon must designate (T=Total, P=Particulate, D=Dissolved); if more than one type of organic carbon is reported in the publication, record T in the field and the other values (P or D) as a Remark; if the value is reported as "organic carbon" without identifying type, assume the value is expressed as Total and report T. Sediment organic carbon values are not reported. |

9. Remark Parameters

The REMARK field contains additional information about a coding field. The coding sheet does not reflect a discreet REMARK field. Reviewers should code remarks in available blank space. Remarks for an AQUIRE field begin with a field name identifier, then a slash (/), followed by text and end with a double slash (//).

Example: Site/LI, KI, MU //

When additional information is necessary for coding a field, a slash is placed in the coded field and a remark field name identifier is placed in the REMARK field to link the remark to the coded field. A complete list of field names is documented in ECOTOX Appendix DD.

10. Field Testing Parameters

Habitat Comment (HABITAT CODE)

In the first box, a one-letter code based on the Cowardin system code (see ECOTOX Appendix X) is used to describe the habitat (eg., Lacustrine or Riverine). The descriptor field is used to record the author's description of the water body, e.g. brackish marsh, oligotrophic lake, plastic tub, polyethylene lined enclosure. If the author does not provide any information about the habitat, both fields are coded as NR (not reported).

| Habitat Code | |
|----------------------------|-----------------------------------|
| <input type="checkbox"/> P | concrete tanks in natural pond |

Substrate (SUBSTRATE)

The bottom substrate is recorded as a two letter code by using the SUBSTRATE codes listed in ECOTOX Appendix Y. If there are no applicable codes, record as the author states in the literature. If a substrate is not reported, NR is recorded. A mixture of sediment types is coded as "MX" and should also include text for the most prevalent soil type(s) in the mixture.

Differentiate between organic and mineral soil/sediment by recording O for organic (leaves, detritus, debris) and M for mineral. Report % organic matter, if reported in literature.

| Substrate | |
|-----------|---------------|
| MX | SA, GR, rocks |

Water Depth (DEPTH)

Water depth value and unit are coded for the study site, as reported by the author. The software will convert the depth to a metric unit. "NR" is coded in the DEPTH field if the author does not report the water depth at the study site. If the author only reports the water depth of the entire system or the depth at which experimental units (i.e., cages) are suspended, "NR" is coded, and depth information is included in the EXP DESIGN field. (See ECOTOX Appendix Z for valid unit codes)

Geographic Location (LOCATION)

Water body, city, county or relevant site information is coded. (see ECOTOX Appendix AA for field location abbreviations.)

Geographic Code (ST/PR/COUNTRY)

This field will contain the state, province or country name of the test site along with the Geo code. If the test site is not reported, an "NR" is coded. (ECOTOX Appendix BB contains a listing of country, region, province names and associated Geo code.)

Longitude/Latitude (LAT/LONG)

If reported by the author the latitude and longitude are recorded. The "~" sign replaces the "" sign in data entry. If not reported, NR is recorded.

An example of a longitude/latitude location (MED, Duluth, MN) is listed below:

Latitude: 46~50'51" N
Longitude: 92~11'12" W

An example of a ranged longitude/latitude location is listed below:

Latitude: 52~30'-53~30' N
Longitude: 107~30'-106~30' W

Application Type (AP TYPE)

This code will contain the method of application of the chemical. Application type codes are located in ECOTOX Appendix J.7.

For instances where the reviewer is unsure whether the chemical was applied directly to the water body by pumping, pouring, metering, etc., "DA" (Direct Application) will be coded.

Application Rate (AP RATE)

This field contains the application rate value and the units that the author reports. If an application rate is not reported by the author, record as NR. If an exposure concentration is not reported by author, the application rate must be reported. (See ECOTOX Appendix N for application units)

Chemical Half-life (HALF-LIFE)

Record the specific chemical half-life in water as measured by the author. If the half-life is referenced from another paper, code this field as NR. If information about the half-life is not reported, record NR.

Example: HALF-LIFE: 2d

Application Season (AP SEASON)

This field is used **ONLY** if no application date is given by the author but the author does specify a season. This field contains the season of initial application of the chemical. A list of application seasons with dates and AQUIRE codes is presented below:

| <u>Code</u> | <u>Season</u> |
|-------------|---------------|
| WI | Jan-March |
| SP | April-June |
| SU | July-Sept |
| AU | Oct-Dec |
| NR | Not Reported |

Application Date (AP DATE)

The application date is the time of initial exposure. The format is mm-dd-yyyy, e.g. 12-01-1993. If one or more parts of the date is not reported, code the letter of the date that is missing (e.g. 12-dd-1993, mm-dd-1993, 06-15-yyyy). If more than one initial date is reported (e.g. more than one pond exposed), record the dates as a REMARK. If one pond is exposed multiple times, only report the first application date and note #x in frequency. If the application date is not reported, NR is recorded.

11. References

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ECOTOX

ECOTOXicology Database System

TERRETOX Coding Guidelines

Prepared for

U.S. Environmental Protection Agency
Office of Research and Development
National Health and Environmental Effects Research Laboratory
Mid-Continent Ecology Division (MED)
Duluth, Minnesota

By

Computer Sciences Corporation
Duluth, Minnesota 55804
Contract 68-W-02-032, Task Order #2024

AUGUST 2003

| | | |
|-----------|---|------------|
| C. | TERRETOX CODING GUIDELINES | C1 |
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| 3. | Test Information | C7 |
| A. | Test Organism Information Parameters | C7 |
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| | | |
|--------------------------------|---|-----|
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C. TERRETOX CODING GUIDELINES

The TERRETOX Coding Guidelines presented below follow the format of the TERRETOX coding sheets located in Section 6. Supporting Materials. The TERRETOX Coding Sheet is divided into four sections: Quality Assurance Parameters, Test Information, Exposure Information, and Results Information. The field name associated with each test parameter, as presented on the TERRETOX Coding Sheet, is a topic heading. Below each heading is a detailed description of how to code data related to the specific test parameters. With few exceptions, reviewers should code the information as presented by the authors. Decisions regarding which information to code and how to represent the information in the database are based on the terrestrial plant and wildlife toxicity testing methods presented in ASTM and US EPA publications, as well as the scientific literature and toxicology textbooks. Test method publications used for the Terrestrial Plant and Wildlife Toxicity Effects (TERRETOX) database are listed in Section 6. Supporting Materials. Questions related to this document should be directed to the EPA Database Coordinator and the Lead Reviewer on the ADP Support Contract.

Note: Inclusion of publications into the TERRETOX database is determined by the test media used. If a terrestrial organism is exposed in an aqueous media, the paper will be placed into the AQUIRE database. Terrestrial nematodes are an example of an organism that may be coded either in TERRETOX or AQUIRE. If the test is conducted in soil media, then the data are coded in TERRETOX; if the nematode is isolated from soils and exposed using aquatic test methods, then the data are coded in AQUIRE. The exception to this rule is for hydroponic studies. Hydroponics is a terrestrial test method and is coded as such.

Note: Each publication included into the TERRETOX database must meet the five minimal criteria for acceptance (i.e. chemical, species, concentration, duration and effect). If the paper is missing one or more of these criteria ECOTOX does not search other sources to obtain the missing data piece(s). Sources such as author communications and referencing another work to obtain one of the five criteria is allowed for specific risk assessment/criteria projects (e.g. EcoSSL or CAD) and must be specified by the EPA Database Coordinator.

Note: Only quantitative data are encoded into TERRETOX. If additional data are represented qualitatively (i.e., no numeric response values), the qualitative effects are noted in the general comments section. If the publication only reports qualitative data, the publication will be rejected.

Note: Lead shot papers are not encoded into TERRETOX. Authors often report the number of pellets fed to the animals, but not the actual dose of lead or concentration of lead/pellet. There are also instances in which the lead pellet is actually a mixture of two metals (i.e. Lead and tin).

Note: If a paper reports that the organisms used for measurements were dead and it is unknown how long the organisms have been dead this data is not encoded, but enter "dead organisms" in Other Effects. An author may measure the growth or weight of a dead organism. The weights of dead animals can be biased, especially for juveniles, depending on the time between death and measurement. Also, temperature may effect the measurement after death (e.g. higher brooder temperatures may cause rapid dessication which would result in weight changes).

Note: Studies involving carbon dioxide (CO₂) or ozone (O₃) as the toxicant are not coded into the TERRETOX

database.

1. Quality Assurance Parameters

Reference Number (#), Author, Year

Reference number is the unique number that identifies a particular publication. This number, automatically assigned by the data entry program, provides the link between data entered and the original publication. On the coding sheet, enter the reference number located in the upper right-hand corner of the hard copy of the publication, the last name of the first author, and the publication year in the data field REFERENCE #, AUTHOR, YEAR.

Total Tests

Total tests encoded for a publication are recorded by the reviewer in the TOTAL TESTS data field. The total test number equals the total number of results records coded in the RESULTS INFORMATION section of the coding sheet for each publication. Total tests are counted after the data abstraction process has been completed.

Reviewer/Review Date

The person conducting the data abstraction enters his/her last name in the REVIEWER data field. The date on which the publication was reviewed is entered in the REVIEW DATE field using the format of month/day/year.

QA Date/Name

Following data coding and prior to data entry, an ECOTOX staff member conducts a screening check of each coding sheet to ensure completeness and accuracy of data transcription. The person conducting this quality assurance screening check enters the date of the QA check in the QA DATE field, using the format of month/day/year, and their initials in the NAME data field.

Test Number (TID)

Each unique test is coded on a separate data sheet and assigned a unique test number (TID) by the reviewer. A unique test design may be characterized by a new test chemical, test species, test location, or exposure type. Additionally, there are experimental design (EDES) parameters that will influence a test scenario sufficiently to warrant an independent Test TID. Such parameters include tests conducted at different test temperatures or conducted during different seasons. Some examples are found in Tables 1 & 2.

Table 1: A study is conducted with 2 different chemicals and the exposure for 2 species is started at 3 different lifestages. The Test IDs would be:

| TEST ID | Unique Test Design |
|---------|-------------------------|
| 1 | Benzene, Worm, Cocoon |
| 2 | Benzene, Worm, Juvenile |
| 3 | Benzene, Worm, Adult |
| 4 | Benzene, Bird, Egg |
| 5 | Benzene, Bird, Juvenile |
| 6 | Benzene, Bird, Adult |
| 7 | Toluene, Worm, Cocoon |
| 8 | Toluene, Worm, Juvenile |
| 9 | Toluene, Worm, Adult |
| 10 | Toluene, Bird, Egg |
| 11 | Toluene, Bird, Juvenile |
| 12 | Toluene, Bird, Adult |

Table 2: A study is conducted with 1 chemical and the exposure for 1 species is conducted at 3 different temperatures. The Test IDs would be:

| TEST ID | Unique Test Design | Exposure Info Remark |
|---------|--------------------|-------------------------|
| 1 | Benzene, Worm, 15C | EDES/Conducted at 15C// |
| 2 | Benzene, Worm, 20C | EDES/Conducted at 20C// |
| 3 | Benzene, Worm, 25C | EDES/Conducted at 25C// |

If appropriate, include information about the Experimental Design parameters in the REMARKS data field for Species Information, Exposure Information or Soil Information as well as in the REMARKS data field for each independent Observed Response value reported.

2. Test Chemical Parameters

ECOTOX is catalogued by the toxicant tested using the Chemical Abstracts Service (CAS)

registry number. If a CAS registry number is not available through standard sources the toxicity data cannot be included in ECOTOX. Additional toxicants not included in ECOTOX are water/soil chemistry effects (e.g., pH), complex effluents, and chemical mixtures.

Chemical mixtures may be interpreted broadly. For example, if a pesticide is a mixture of two active ingredients, each may have a separate CAS number. However, if the formulation of the two ingredients has a CAS number, the chemical reported for ECOTOX is the formulation. If the exposure is based on two metal compounds but the effect is based on one ion, e.g., copper sulfate and copper chloride and Cu is the toxicant, code copper as the test chemical and report the two compounds in chemical comments.

Another differentiation of a mixture is drawn when nutrients are added to an experimental set up. If the author states that a nutrient is added at a level that is needed for growth, ECOTOX does not consider this as a mixture. If, however, the author adds a series of nutrients and toxicants to test interactive effects, ECOTOX considers this a mixture. The following example illustrates how nutrients and toxicants are coded.

A. Effect of copper on plant growth

| Nutrient Copper | 20 ug/l | 40 ug/l |
|--------------------|---------|---------|
| 0 ug/l | 100% | 120% |
| 10 ug/l | 100% | 90% |
| 20 ug/l | 80% | 80% |
| 30 ug/l | 60% | 50% |
| 40 ug/l | 30% | 20% |

B. Effect of copper and nutrient on plant growth

| Nutrient Copper | 0 ug/l | 10 ug/l | 20 ug/l | 30 ug/l | 40 ug/l |
|--------------------|--------|---------|---------|---------|---------|
| 0 ug/l | 100% | 100% | 100% | 110% | 120% |
| 10 ug/l | 100% | 100% | 100% | 100% | 90% |
| 20 ug/l | 95% | 90% | 80% | 85% | 80% |
| 30 ug/l | 60% | 60% | 60% | 65% | 50% |
| 40 ug/l | 20% | 15% | 30% | 35% | 30% |

A. Author states that nutrients are added for growth. All results coded. Each nutrient level is coded as a separate test and the nutrient level is noted in an experimental design set-up (EDES) comment.

B. Author does not state that nutrient is added for growth. Two tests are coded, one for the nutrient tested alone and a second result for the copper tested alone. The shaded area is not coded. Mixture is noted in general remarks.

If the author does not document the basal level value, a determination may be possible for deficient, basal and toxic dose levels. The suggested guideline for making this determination would be interpreting dose response results for mortality, growth and reproduction to determine the deficient, basal (or basal range) and toxic levels. This could be accomplished by interpreting the trends for these effects. The deficient results would be excluded and basal level are coded

as the control. If multiple basal values are reported, the most optimal dose for mortality, growth, reproduction would be considered the control value.

If the toxicant added does not produce a toxicity test result (i.e., only deficient and/or basal levels), then the paper would be rejected as nutrient study.

For *in situ* exposures where the exposure is by default an exposure to a chemical mixture; code residue measurements or endpoints (BCF) only. No other effects or endpoints are strictly attributable to a single chemical in the same way as a residue concentration. Data for chemicals in the mixture with reported media concentrations and residue effects should be coded.

A standardized identification number and name for each chemical is recorded in the database to ensure quality and consistency. Toxicants, carriers and positive control chemicals reported in ECOTOX are assigned a Chemical Abstract Services (CAS) Registry number and are referred to by the Collective Index (CI) standard nomenclature. The CAS number and CI name are stored in a chemical card file and an online index file (EcoChem). EcoChem is available for screening CAS numbers and chemical names used in ECOTOX. Chemical name synonyms are stored electronically, but are also available from the chemical card file.

Test/CAS Number/Chemical Name/

Record the test, carrier and/or positive control chemical name as it is reported in the publication. The test chemical, as presented by the author, is reported on line number one (TEST). The CAS number is assigned by locating the chemical name in the ECOTOX chemical card file. If the chemical name is not in the chemical card file, record a 'no' in the CAS number field and the coding sheet will be referred to ECOTOX staff for CAS number verification following completion of the coding and screening quality assurance checks.

For the remaining chemical information lines, record the chemical name as reported by the author regarding any carriers, solvents or positive controls used for the test. If neither a carrier/solvent nor a positive control was used, report as 'NR'. If a carrier/solvent or positive control was used, circle "Carrier" or "Positive Control" as applicable. Frequently used carrier/solvent CAS numbers are listed in Appendix A. The CAS numbers for positive control chemicals are assigned by locating the chemical name in the ECOTOX chemical on-line or card file. If the chemical name is not in the chemical card file, record a 'no' in the CAS number field and the coding sheet will be referred to ECOTOX staff for CAS number verification following completion of the coding and screening quality assurance checks.

Note: Water should not be coded as a solvent. A solvent is defined as an agent (other than water) in which the

test chemical is mixed to make it miscible with dilution water before distribution to test chambers. Solvents or carriers are used in toxicity tests where the concentrations of the test chemical are extremely low and a very small amount of test material must be added to the test chambers. (Rand, 1995)

Note: Exposure and observation data for carrier and positive control chemicals are reported in the Exposure and Results sections. Refer to these sections for specific instructions.

Chemical Grade

Record the chemical grade information for each chemical reported in the GRADE data field (refer to Appendix B for the applicable codes).

Chemical Purity

Record the numeric percentage information about the purity or active ingredient of the chemical in the PURITY data field (e.g., if the author reports 97% purity, 97 would be entered into this data field. PU for purity would be entered into the FORMULATION data field (see CHEMICAL FORMULATION).

Chemical Formulation

Record the chemical formulation code for each chemical reported in the FORMULATION data field (refer to Appendix C for the applicable codes).

Chemical Comment

Supplemental information about the test chemical is entered into the CHEMICAL COMMENT field. If a mixture of labeled and unlabeled chemical is used, remark "labeled and unlabeled" in this field. Record additional relevant chemical information such as trade names, common names, or isomers in this field.

Radiolabel

If a radiolabeled chemical is tested, record the isotope in the RADIOLABEL field (see Appendix D for codes). When the specific isotope is not reported or when multiple isotopes are reported, the field is marked with an asterisk (*). In REMARKS, note either RADIO/no isotope reported// or RADIO/isotope xx and isotope yy//. When both radiolabeled and unlabeled test chemicals are used in a test, report the radiolabeled isotope and code "labeled and unlabeled" in the

CHEMICAL COMMENT field.

Note: Any REMARKS made for fields in this section will be recorded according to the instructions set forth in Test Information.

Chemical Abstracts Services Registry Number (CAS NUMBER)

The CAS Number of the toxicant is recorded in the CAS NUMBER field. A standardized identification number and name for each chemical recorded in the database is used for consistency. Toxicants included in the ECOTOX database are assigned a CAS registry number and are referred to by the Ninth Collective Index (CI) standard nomenclature. The CAS number and CI name are stored in a chemical card file and in an online index file (ECOCHEM) which is available electronically for screening CAS numbers and chemical names used in ECOTOX. If a hydrated form of a chemical is used in the paper, record the hydrated form as reported by the author in the TEST field. However, record the CAS Number for the non-hydrated form of the chemical in the CAS NUMBER field.

3. Test Information

This section is used to report general information describing the test scenario. If any of the following information changes, a new Test ID is assigned and a new coding sheet is required. Specifically, the Test Information section describes the test organism, the test location and exposure type, information about the type of controls used, the total number of doses, and the application frequency. Refer to Table 3 for coding examples.

A. Test Organism Information ParametersSpecies Number/ Scientific/Common Name

The test organism is identified by the current scientific name as verified in the taxonomic literature. Enter the species name, as presented by the author in the SPECIES SCIENTIFIC/COMMON NAME field. Each unique test organism is assigned a species number which is stored in the CRITTERS database. Locate the number for the species in the CRITTERS database and enter it in the SPECIES NUMBER field. If the species is not in the CRITTERS database enter 'no' in the SPECIES NUMBER field, and the coding sheet will be referred to ECOTOX staff for species verification following completion of the coding and screening quality assurance checks. For each species number, the verified name, taxonomic hierarchy, nomenclature history, and verification sources are kept on file for quality assurance documentation.

Generally, when coding effects in ECOTOX, the data are reported for each individual species. Field studies may report results for a target community (e.g., beneficial and non-beneficial insects) or for an entire enclosed ecosystem (e.g. system-level primary productivity or respiration). Assign a community to the most specific taxonomic level possible (e. g. "earthworms" would be classified as "Oligochaeta," "weedy plant species" would be classified as "Magnoliophyta."). If you are not sure about the classification of a community, enter 'no' in the SPECIES NUMBER field, and it will be sent to ECOTOX staff for verification.

Decisions regarding the inclusion of species in TERRETOX are based on published terrestrial ecotoxicology standard methods and procedures documentation (eg., Menzer et al 1994; US EPA testing series; ASTM testing series). The focus for TERRETOX is to collect publications with data for soil invertebrate and microbial species, plant species (agricultural and native), wildlife avian species (e.g. mallard, pheasant or bobwhite), wild mammalian species (e.g., meadow vole, deer mouse or mink), terrestrial lifestages of amphibians and reptiles, and beneficial invertebrate species (e.g., honey bee, leafcutter bee or alkali bee). If data for other species including laboratory, domestic or non-beneficial organisms are reported in a publication, data for all test species are coded for entry into TERRETOX. Publications focusing primarily, or solely, on laboratory, domestic or non-beneficial organisms are not actively acquired or coded at this time.

Organism Source

Report the source of the test organism in the ORGANISM SOURCE data field (see Appendix E for codes). The source explicitly includes the strain of the organism, e.g. laboratory strain versus wild strain.

Organism Lifestage/Age

The LIFESTAGE/AGE data field records the specific lifestage and/or age for each test organism at beginning of exposure, as reported in the paper (see Appendix F for lifestage codes and Appendix I for time units associated with the age of the organism). Record the lifestage information in the first box and age information in the second box on the coding sheet. Record as 'NR' if the information is not reported in the publication.

Organism Comment (Org Characteristics)

Report any general information provided about the test organism. Characteristics may include information such as specific strain name, cultivar, variety, weight, length, developmental stage, hybrids or taxonomic groupings used to describe the organism being tested.

Note: Information regarding the sex of the test organism is coded in the Sex field, see Exposure Information. The sex of the organism is often directly linked to the exposure and subsequent response observations; for example, specific reproductive responses are unique to males or females.

Note: When reporting a cultivar, include 'cv.' before the name of the cultivar. Include 'var.' for variety in a similar manner.

Table 3. Test Information Coding Sheet Example

| | | | |
|---|-------------------------------|-------|--|
| SPECIES SCIENTIFIC/COMMON NAME __Aphis sp. _____ | | | ORGANISM INFORMATION |
| SPECIES NUMBER | 5519 | | |
| ORGANISM SOURCE | WLD | | |
| LIFESTAGE/AGE | NR | 1-2 d | |
| CHARACTERISTICS | A. mellifera and A. ligustica | | |
| TEST LOCATION | LAB | | EXPOSURE INFORMATION |
| EXPOSURE TYPE | FD | | plants sprayed outdoors in evening; moved to lab next day; bees exposed to plants in lab |
| EXPOSURE DURATION | 6 D | | |
| STUDY DURATION | 2 WK | | |
| CONTROL TYPE | B | | |
| NUMBER OF DOSES | 3 | | |
| APPLICATION FREQUENCY | ADL | | |
| MEDIA TYPE | NAT | | SOIL INFORMATION |
| SOIL TYPE | Pedozioic Clay -silt | | |
| SOIL TEXTURE % | SA 79 SI 15 CL 6 | | |
| MEDIA PH | 5.6 | | |
| MEDIA ORGANIC MATTER | 5 % | | |
| MEDIA MOISTURE (%) | 31 | | |
| MEDIA CEC | NR | | |
| SOIL CONC MEASURED / DRY-WET WEIGHT | M | DRY | |

B. Exposure Information Parameters

Test Location

Report the location or setting in which the experiment was conducted in the TEST LOCATION data field (see Appendix H). For example, a natural field study (FieldN) is an experiment conducted outdoors in a natural setting. The test organisms are sampled in the wild, e.g. population counts. Outdoor studies conducted in a simulated environment are coded as an artificial field study (FieldA). Artificial field studies include organisms isolated from their natural environment via an enclosure of some type, e.g. cages or fencing. If the publication does not provide enough information to distinguish between FieldA and FieldN, then use the code FieldU to indicate that the field test type is unknown. Laboratory tests (LAB) are conducted indoors under controlled laboratory conditions. If the location or setting cannot be identified as laboratory or field from the publication, code as Not Reported (NR).

Exposure Type

For the TERRETOX database, the term 'exposure' is used to refer to the mechanism by which the toxicant was applied. Organisms are typically exposed to toxicants through diet, injection, inhalation, topical or environmental routes. On occasion, an exposure may be through multiple routes (e.g., such as topical and oral).

Some exposures could be coded a variety of ways. For example, exposure as an aerial spray to a field plot could be coded either as a spray application or as exposure through multiple routes, eg. topical (through skin) and diet (from consumption of exposed vegetation) for animals, or topical (through leaves) and environmental (root uptake) for plants. Within the TERRETOX database, this instance is coded as a spray application. Multiple exposure route coding is applicable when the organism is exposed through two *independent* applications, for example, a contaminated diet *and* toxicant inhalation for animals or contaminated soil *and* leaf spray for plants. In this scenario, 'MU' would be entered into the EXPOSURE TYPE data field and a remark (TYPE/'FD' and 'IH'// or TYPE/'PR' and 'FS'//) would be noted in the Exposure Info comments section.

TERRETOX does not include in vitro assays [i.e. an experimental trial, involving biological matter, which is exposed to a toxicant in an artificial apparatus rather than within a living organism] in the database. Studies in which the living organism is exposed as a whole, but an effect on an internal process is examined outside the body after the exposure, are coded (e.g. enzyme functions). The database contains some studies using excised organs and cell cultures from plants, however these types of studies are not actively coded at this time. Future coding of these studies is under discussion.

When coding, report the specific exposure type, e. g., for an intercutaneous injection, code as IC (intercutaneous) not I (injection). For an environmental exposure, code as HS (hand spray) not V (environmental). If an exposure type is not reported, code as Not Reported (NR). Refer to

Appendix J for exposure type codes.

Control Type

Effects of toxicant exposure are evaluated by comparing the exposed organisms to untreated organisms - the controls. All toxicity tests should include a concurrent control where the test conditions are identical except for the absence of the toxicant. Some toxicity tests will also include a control for other test conditions such as the use of a solvent, feeding or acclimation requirements, historical or pre-exposure conditions and for establishing reproducibility by use of a reference toxicant. (Doull et.al. 1980)

Report the type of test control(s) used in the study (Appendix M) by recording the applicable code in the CONTROL TYPE field. If more than one type of control is used in the study, e.g., a dilution water and carrier control, code 'M' for multiple controls. Often comparisons are made that do not meet the criteria for a control; these types of comparisons include starvation studies and acclimation periods. Report the studies that complement the toxicity test, e.g. a starvation study used in a feeding behavior or avoidance test, as a comment in the REMARKS data field in the Test Information section of the coding sheet. Sometimes a paper will report a table of baseline or historical control values. Do not code these values unless there is a direct correlation to a measurement or endpoint; code only control values which complement response values.

When data for the control are reported only in graphical format, interpret the data as accurately as possible and remark that the control data were obtained from a graph in the results information section. Data points derived from a graph are most typically represented as an approximation of the data point, a range around the specific data point or as a range for all of the represented values.

If a control is identified for the test but no exposure or results data are reported, record the appropriate control type code in the Control Type field. No data will be coded in Exposure or Result Information fields.

Number of Doses

Report the total number of exposure doses, including the controls, for each independent test design in the NUMBER OF DOSES data field. If number of exposures is not reported, e.g. in a publication reporting only calculated endpoints such as LD50s, code the field as 'NR'. Do not include endpoint or ranged doses or number of replicates in the total number of doses.

Application Frequency

Report the frequency of the dose application in the APPLICATION FREQUENCY data field. Refer to Appendix K for application frequency codes and units.

Exposure and Study Durations

A toxicity test may range in duration from a pre-treatment period through the actual toxicant exposure and conclude with observations of the organisms post-exposure. Duration information is coded using the units reported in the publication (see Appendix I for valid units). Refer to Table 4 for a coding example. Exposure and study durations are reported with the Test Information. Observation Duration is reported with the Results Information.

Table 4. Example 17-day experimental period with 2-day pre-treatment, 5-day exposure, and 10-day observation.
Note: Pre-treatment days are not included in the study duration.

| | DURATION OF EXPERIMENT | | | | | | | | | | | | | | | | |
|--------------------------------|------------------------|----|----------|---|---|---|---|-------------|---|----|-----|----|----|----|----|----|----|
| | Pre-Trt | | Exposure | | | | | Observation | | | | | | | | | |
| Calendar Days | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11* | 12 | 13 | 14 | 15 | 16 | 17 |
| Test Periods | 1 | 2 | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Reported Days (Study Duration) | -2 | -1 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |

Note: In test scenarios where incubation times are reported, i.e. enzyme fixation assays, be careful to report the toxicant exposure time *not* the assay incubation time.

Exposure Duration

The exposure duration is a mandatory field for inclusion in the TERRETOX database. In cases where the observation time is the only duration reported, it is assumed that the exposure duration is equivalent to the observation time. If the exposure duration is not reported, the paper is rejected. The period of time recorded in the EXPOSURE DURATION data field is the time of actual exposure to the chemical. For example, in Table 4 the exposure duration equals 5D.

In some cases a biological time is used, such as an exposure time reported as "until hatch", "growing season" or "after the nth egg has been laid". Use the code from Appendix I that best describes the author's words in duration units data field. 'NA' will be coded as the duration mean value for all biological time durations (e.g. EXPOSURE MEAN: NA, EXPOSURE UNIT: HT; corresponds to an until hatch duration), unless a numeric value is substituted (e.g. EXPOSURE

MEAN: 2, EXPOSURE DOSE: HV; corresponds to a 2 harvests duration). However, references to time such as "observed at end of the study period" are not coded; such papers are rejected as having no exposure duration.

For injection, diet, topical and environmental exposures where the actual exposure is dependent on biological and environmental conditions, the exposure time is recorded as equivalent to the study time. This assumption is made to ensure consistency in data representation; it is not necessarily a true reflection of the exposure time.

Study Duration

The study duration is the total time of the study *excluding* pre-treatment times. In the example in Table 4, the study duration is equal to 15D (5D exposure plus a 10D observation). In cases where the observation time is the only duration reported, it is assumed that the study duration is equivalent to the observation time. The study duration will be reported as 'NR' if no observation or study time is reported.

Note: for most field studies the exposure and study duration are identical because it is difficult to determine when the exposure ends. It is difficult to know when the application has completely dissipated in the environment. For lab studies the exposure and study duration may be different. This difference will be seen when there is a recovery period from exposure duration. For lab studies when the treatment is some type of injection or diet (intraperitoneally or by gavage), study duration and exposure duration are the same.

Media Type

Report the type of exposure media, (e.g., natural or artificial soil, aqueous (hydroponic), filter paper), in the EXPOSURE MEDIA data field using codes presented in Appendix L. Report as 'NR' if you cannot determine the exposure media from the paper. If an aqueous exposure is conducted in pore water from a specific soil, report the soil parameters in the soil characteristics fields (pH, CEC, OM, etc.). If the bottom of the experimental chamber is covered with sand and then topped with filter paper, an Experimental Design (EDES) remark should be made that sand was used in the chamber, but MEDIA should be FLT.

C. Media Information Parameters

Soil Type

Report the scientific name of the natural soil or commercial name of the artificial soil used in the study in the SOIL TYPE data field. If the scientific name is not included report the type of soil using the author's terminology, eg., forest soil, sandy loam soil, arboreal coniferous soil.

Soil Texture (Sand, Silt and Clay)

Report the texture of the soil as stated by the authors in the SOIL TEXTURE data field using percentages of sand (SA), silt (SI) or clay (CL).

Note: Clay may be reported as bentonite, kaolinite or montmorillonite.

Media pH

Report the pH of the test media used in the MEDIA pH data field. If the pH of the treated media is not presented, but the pH value is stated for the untreated or acclimation media, code the untreated media pH and add an asterisk to the end of the value. If the author specifies a measurement method for the pH value (e.g., that the pH value is measured by pHKCl or pHCaCl₂), code the pH value and identify the measurement method in the REMARKS field. If the authors report that a standard (see Attachment G for list of standard soils) or commercially available artificial soil is used, but do not present pH, use the pH reported in the standard test method referenced by the author. If pH is reported for the untreated or acclimation media, code this pH value in the same way as outlined previously and denote with an asterisk. If the pH of a specific soil type is not given in the publication, a search of the USDA/NRCS National Cooperative Soil Survey (USA) online site, at the following web address:

<http://www.statlab.iastate.edu/cgi-bin/osd/osdname.cgi> or the United States Department of Agriculture's Natural Resources Conservation Service National Soil Survey Center site at the following web address: <http://vmhost.cdp.state.ne.us:96> can be conducted for the specific soil series. If the pH is found, range the pH values for all soil depths in the pH data field and remark in the comments section pH/from USDA web source//. Attach a printout of the pH information from this site to the publication.

Media Organic Matter Type and Units

Report information about the test media organic matter as presented by the author. Use the measurement value, organic matter type, and units reported by the author. Refer to Appendix FF for organic matter type codes and units. If carbon and/or nitrogen content of the soils are reported, record these values in the Soil Information Remarks section; organic matter content may be estimated from these values. If the authors report that a standard (see Attachment G for list of standard soils) or commercially available artificial soil is used, but do not present organic matter content, use the organic matter content reported in the standard test method referenced by the author. If organic matter is reported for the untreated or acclimation media, code this organic matter value in the same way as outlined previously and denote with an asterisk. If the organic matter of a specific soil type is not given in the publication, a search of the USDA/NRCS National Cooperative Soil Survey (USA) online site, at the following web address:

<http://www.statlab.iastate.edu/cgi-bin/osd/osdname.cgi> or the United States Department of

Agriculture's Natural Resources Conservation Service National Soil Survey Center site at the following web address: <http://vmhost.cdp.state.ne.us:96> can be conducted for the specific soil series. If the organic matter is found, range the organic matter values for all soil depths in the OM data field and remark in the comments section OM/from USDA web source. Attach a printout of the organic matter information from this site to the publication.

Media Moisture

Report percentage of moisture in the test media in the MEDIA MOISTURE data field. If moisture is reported for the untreated or acclimation media, code this moisture percentage and denote it with an asterisk.

Media Cation Exchange Capacity (CEC)

Report cation exchange capacity and units (Refer to Appendix FF for organic matter type units) of the test media in the MEDIA CEC data field. If the cation exchange capacity is reported for the untreated or acclimation media, code this value and denote with an asterisk.

Soil Concentration (CONC) Measured / Dry-Wet Weight

If soil was the exposure media, use the first data field to report if the toxicant concentration was measured in the soil. If measured, code as 'M' in the SOIL CONC MEASURED data field. If not measured or no information is provided, code as 'U' or 'NR' respectively in the SOIL CONC MEASURED data field.

For instances where some treatment levels are measured and some are unmeasured/nominal, denote the SOIL CONC MEASURED as 'X,' signifying that a mixture of measured and unmeasured values was reported in the publication, but ECOTOX reports the nominal concentrations.

Soil Concentration (CONC) Basis of Measurement: Dry/Wet Weight

Record whether soil concentration was reported based on dry or wet weight in the DRY - WET WEIGHT data field.

Media Comment

Test Information REMARKS sections are used to include additional information necessary for interpreting any of the specific test information fields as well as for providing information concerning the test in general. When additional information is necessary for a given field write 'FIELD NAME/remark text/' (refer to Appendix EE for applicable field name abbreviations). For general information that is not associated with a specific field, label the Remark as Other Effects (OEF). The Experimental Design (EDES) notation is used to identify information that differentiates between exposure scenarios but does not directly implement changes in the data fields. Information that may make a significant change in test design includes varying exposure substrates or seasonal exposure scenarios.

4. Exposure Information

This section is used to record the exposure parameters for each specific test. A specific test is identified by the Test ID (TID) number as previously described. Within each specific test, information is recorded for every treatment level including test controls, positive controls, carrier controls, and toxicant exposures. Such information includes the sample number and sex, the exposure dose, whether the dose is reported in ionic form, the chemical analysis method, and any pertinent remarks (see Table 5).

Dose ID and Dose Number (No.)

Each treatment in a test is assigned a Dose ID and a Dose Number. Controls are always reported first and identified by the appropriate letter code from Appendix M in the DOSE ID field. Exposure doses are identified by the letter 'D'. A link is created to calculated endpoints that are dependent on multiple exposure doses by coding a line identified by the letter 'E' (the linkage 'E' is not used for BAF, TKNO or LTxx/ETxx data. These are linked to a specific dose). Information taken from a graph (responses that DO NOT have endpoints or statistics) may be coded using a ranged dose 'R' that encompasses all of the exposure concentrations excluding the control(s). If more than one type of control is used in the study, e.g. a dilution water and carrier control, code two lines for control, ('C' for the dilution water control and 'V' for the carrier control) in the DOSE ID field. If more than one control of a specific type is used, number each control in the set as a replicate, e.g. 1V, 2V. If a control or treatment is identified for the test but no exposure data are reported, there will not be any data to code for Exposure Information or Result Information. See Tables 3, 4 and 5 for coding examples.

When replicates are used *and* the results are reported separately for each replicate, code a separate line for each replicate. When the publication notes that replicates were run but the author *only* reports the results as the mean of the replicate values, do not code individual lines for the replicates but instead note this information in General Remarks, ie. "x replicates;" see also the Observed Response Value section in Results Information for additional instructions.

When dose data are reported only in a graphical format, interpret the data as accurately as possible and remark that the data were obtained from a graph. Data points derived from a graph are most typically represented as an approximation of the data point, a range around the specific data point or as a range for all of the represented values.

Note: For the example in Table 5, the Number of Doses reported in Test Information would be six (6) to represent the two control levels and four treatment levels; all doses tested are recorded in this field regardless of whether responses are reported. Endpoint (E) and range (R) "doses" and replicate concentrations DO NOT get counted in the total number of doses.

Table 5. Exposure Information

| DOSE No | DOSE ID | N | SEX | DOSE | SM | VALUE | UNIT | ION | M/U | RN |
|---------|---------|----|-----|------|----|-------|------|-----|-----|----|
| 1 | C | 10 | F | 0 | - | - | ppm | - | U | 1 |
| 2 | C | " | " | " | - | - | " | - | " | NR |
| 3 | V | " | " | 1 | - | - | ug/l | - | M | NR |
| 4 | D | " | " | 3 | SE | 0.01 | ppm | Cu | " | 2 |
| 5 | D | " | " | " | " | 0.15 | " | " | " | " |
| 6 | D | " | " | 9 | " | 0.02 | " | " | " | " |
| 7 | D | " | " | " | " | 0.15 | " | " | " | " |
| 8 | E | NR | " | NR | NR | NR | NR | " | " | NR |

| DOSE No | DOSE ID | N | SEX | DOSE | SM | VALUE | UNIT | ION | M/U | RN |
|--|---------|---|-----|------|----|-------|------|-----|-----|----|
| GENERAL REMARKS 1. CNTRL/Control for first generation only// 2. DOSE/Conc reported as flower residues// | | | | | | | | | | |
| DATA CONTINUES ON NEXT PAGE | | | | | | | | | | |

Note: On occasion, when coding data for the Exposure Information section, the number of test exposures and/or replicates will exceed the allotted coding space. If this should occur, continue coding on a second sheet. Note at the bottom of the exposure information section on the first page that the data continues on a second page.

Note: When concentrations are not reported for soil and pore water doses, but endpoints are reported, code exposure information as 'E-dose# = NR', then code two separate endpoints for the soil and pore water endpoints in the results section. Add a remark RVALUE/soil conc// or RVALUE/pore water conc// respectively.

Sample Number (N)

Sample number, denoted by an 'N' on the coding sheet, reflects the sample size at each exposure dose, i.e., the number of test organisms per treatment. Code as 'NR' if not reported.

Gender (Sex)

This field identifies the sex of the organism (male (M), female (F) or both (B) at each exposure level. The importance of this field becomes apparent where organisms of both sexes are exposed at a given treatment level, but the observations are conducted on either the male or female. In this instance, the SEX field would be coded as B in Exposure Information, with individual results reported for M and F in Results Information in the Sample Number Unit field. See Results Information and Table 5 for coding examples. Code 'NR' if not reported.

Dose

Report the exposure dose as reported in the publication. Report the approximation (~), minus (-), greater than (>), or less than (<) symbols used by the author(s) to describe the exposure dose. The mean and/or range is coded in the DOSE data field and the unit in the UNIT field, see below.

If the range values are confidence interval (CI), confidence limits (CL) or fiducial interval (FI) code the abbreviation in the SM data field. See the coding example presented in Table 5 .

For instances where some treatment (dose) levels are measured and some are unmeasured/nominal, and all unmeasured/nominal concentrations are reported, report the unmeasured/nominal concentrations for each treatment level so that the range of concentrations is consistent and monotonical. Denote all of the concentration analysis methods as 'X,' signifying that a mixture of measured and unmeasured values was reported.

Note: If chemical concentrations (especially metals) are reported in terms of Total, Exchangeable, Water Soluble and Pore Water concentrations, Total is the concentration selected for entry into the dose data field. The other concentrations are reported as remarks.

Note: If a background concentration is reported for the chemical being applied, report the background value in the control dose in the DOSE field.

Dose Statistical Method (SM)

Report the method used to determine the range around the Dose in the SM data field, if reported by the author(s). Use standard codes for the methods, i.e., standard deviation (SD), standard error (SE), confidence interval (CI), confidence limits (CL) or fiducial interval (FI) or range (R). If the interval around a value is not identified in the paper as SD, SE, CI, CL, FI or R, then code as not reported (NR).

Dose Value

Report the numeric value of the standard deviation or standard error around the Dose in the VALUE data field, as reported by the author(s).

Dose Unit

Report the measurement unit that corresponds to Dose in the UNIT data field (see Appendix N for standard units).

Ionic Fraction

For ionizing substances (e.g., metals, ammonia), report the dose as the ion if the concentration presented by the authors is reported as based on the ionic form of the compound (e.g., organotin as Sn). Code the appropriate ionic symbol in the ION data field (see Appendix O for ion codes). If concentration is based on the total compound, code 'NR' in this field. For non-ionizing substances, code 'NR' in this field.

Chemical Analysis Method (M/U)

The M/U data field identifies whether nominal or quantified exposure dose values were reported by the author(s). For the specific exposure level, report whether toxicant and/or carrier concentration was measured (M) or calculated/nominal/unmeasured (U) (see Appendix P for codes and definitions). When it is not clear whether reported concentrations are measured, calculated or unmeasured, record as Not Reported (NR).

For instances where some treatment (dose) levels are measured and some are unmeasured/nominal, and all unmeasured/nominal concentrations are reported, report the unmeasured/nominal concentrations for each treatment level so that the range of concentrations is consistent and monotonical. Denote all of the concentration analysis methods as 'X,' signifying that a mixture of measured and unmeasured values was reported.

Remark Number/Remarks (RN)

When there are remarks for a specific test, the REMARKS field as well as the Remark_Number RN (remarks number) data field, will be coded. Remarks are identified by the coding field abbreviation listed in Appendix EE. The Remark Number (RN) field is used to link the remarks associated with each specific test. Each unique Remark is assigned a Remark Number, and only one Remark Number is used per result entry. Use an independent unique Remark Number for each section of the database, i.e., do not carry over Remarks or Remark Numbers from the Exposure section to the Results section. Refer to Tables 3 and 5 for coding examples.

General Comment

General information about the exposure such as any specific methodology or techniques used is recorded in the REMARKS data field with the Other Effect (OEF) identifier. General information about the test may include names of other chemicals that were tested but were not coded for TERRETOX, results are not provided, effects that have been reported but are not linked to a

dose, effects that are reported but are not applicable to TERRETOX (e.g. in vitro studies, selectivity ratios, acute to chronic ratios), or effect modifiers such as changes in soil pH, temperature or humidity.

5. Results Information

This section is used to record observed effects for each control and dose level reported for the specific test. The Dose ID and Dose No. is carried forward from Exposure Information. Information specific to the observed response includes the sample number and sample unit, exposure duration, descriptors of the effect observed, the response site, and a quantitative measure of the response. Refer to Table 6 for specific fields included in this section of the TERRETOX Coding Sheet.

General Information on Results Coding:

Coding Data Similarly Presented

Often data are reported as individual measurements as well as a mean or range for these values. Report individual test results only. However, if both raw data and percentages, e.g. number survived and % survival, are reported, both values are coded. An exception to this coding procedure occurs when data reported for individual endpoints are graphed and mean/median data are explicitly reported. For example, when replicate LC50s are reported on graphs and the mean and median LC50s are reported in the text, code both the graphed and textual data (remark on data points taken from the graph, and also note mean LC50 or Median LC50 in the comments).

Graphed Data

Data points derived from a graph are represented in TERRETOX as an approximate value, a range around the specific data point or as a range for all of the represented values. The values taken from the graph must be ranged using the author's X- and Y- axis values. Do not interpolate values that lie between axis values. A result remark is added to the observed response field denoting that the data were extracted from a graph.

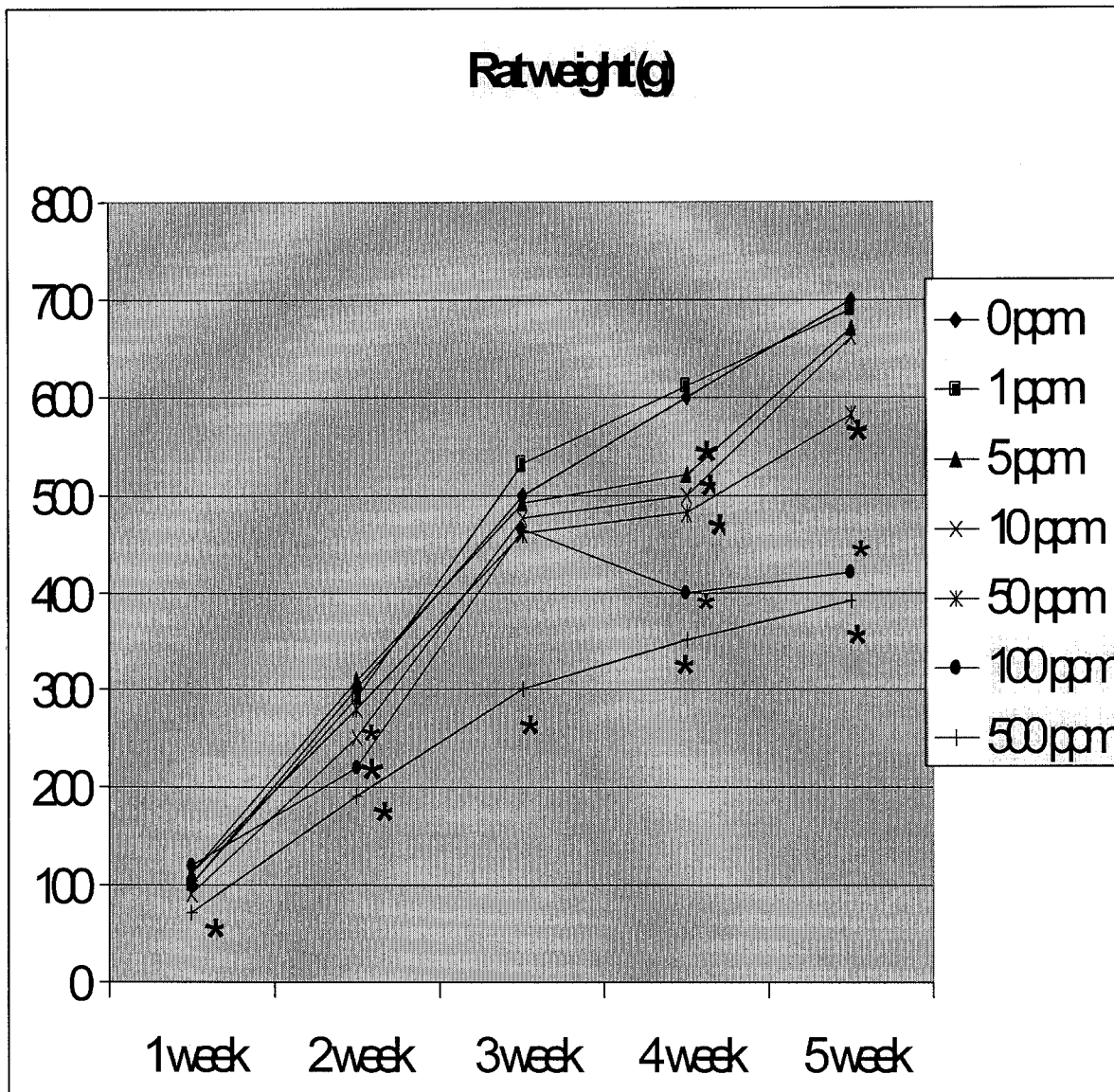
Graphed data are coded one of the following ways:

1. Calculated endpoints (e.g. LC50s, BAFs) are always coded separately.
2. Statistically analyzed graphed data that is graphed is coded as denoted in Example 1. Data trends should be examined (i.e. areas where the exposure changes from not significantly different to significantly different from the control values) and coded. The maximum number of result records that can be coded from statistically analyzed graphed data is $2n-1$, where n is the number of concentrations including the control. If all data points for a single dose are significant, non-significant or have multiple significance (i.e. no clear response) combine all durations into

a single record (see Example 1, doses 2D, 4D and 7D). If a dose presents a clear trend (e.g. non-significant for the first three weeks and significant for the last two weeks) code two results for the dose. Combine the durations for the first three weeks and code the Statistical Significance as "nosig" for the first result and combine the durations for the second two weeks and code the Statistical Significance as "sig". Code records similarly if part of the dose is significant or not significant and the other part has multiple significance (See Example 1, doses 3D, 5D and 6D).

3. For data that is not statistically analyzed, two lines are coded, one line for the control and a second line for all doses. For the ranged doses, the exposure number would be coded as #R (# representing the next dose level) and the exposure concentrations would be ranged in the Dose data field. See Example 2 for the coding of non-analyzed data.

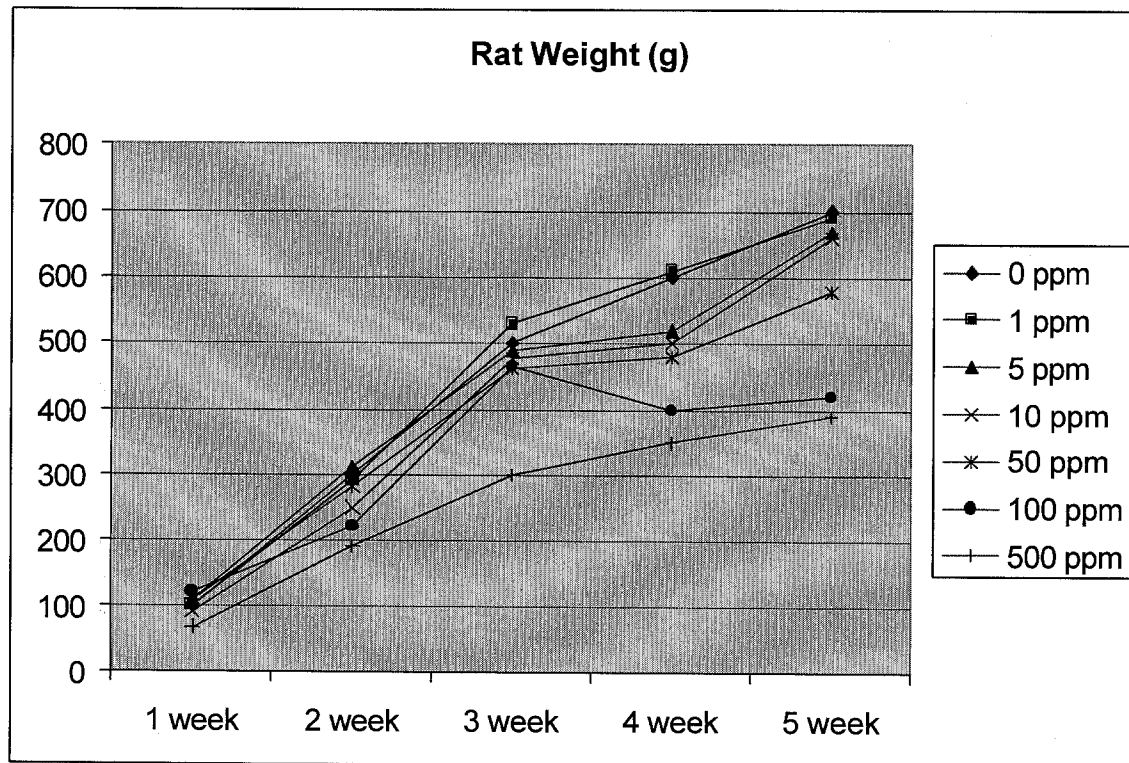
Example 1 Statistically Analyzed Data



Terretox Results Coding for Example 1

| ID | SMP # UNITS | OBSERV DUR | EFFECT | MEAS MENT | END PNT | R | STAT | LEVEL | P | SITE | OBSERVED RESPONSE VALUE/UNIT | | | | | D W % | REMARK |
|----|----------------|---------------|--------|--------------|------------|--------|-------|--------|---|--------|------------------------------|-------------------|--------|--------|------|-------------|----------------------------|
| | | | | | | | | | | | MEAN | RANGE | SM | VALUE | UNIT | | |
| 1C | NR OR | 1-5 w | GRO | WGHT | NR | - 1 | NR | NR | P | W O | N R | ~100/ - ~700/ | N R | N R | g | N R | 1. RVALUE/ FROM GRAPH// |
| 2D | NR OR | 1-5 w | GRO | WGHT | NR | - 1 | nosig | p<0.05 | P | W O | N R | ~100/ - ~700/ | N R | N R | g | N R | 1. RVALUE/ FROM GRAPH// |
| 3D | NR OR | 1-3 w | GRO | WGHT | NR | - 1 | nosig | p<0.05 | P | W O | N R | >100/ - <500/ | N R | N R | g | N R | 1. RVALUE/ FROM GRAPH// |
| 3D | NR OR | 4-5 w | GRO | WGHT | NR | - 1 | mult | P<0.05 | P | W O | N R | >500/ - <700/ | N R | N R | g | N R | 1. RVALUE/ FROM GRAPH// |
| 4D | NR OR | 1-5 w | GRO | WGHT | NR | - 1 | mult | P<0.05 | P | W O | N R | >0/ - <700/ | N R | N R | g | N R | 1. RVALUE/ FROM GRAPH// |
| 5D | NR OR | 1-3 w | GRO | WGHT | NR | - 1 | nosig | p<0.05 | P | W O | N R | >100/ - <500/ | N R | N R | g | N R | 1. RVALUE/ FROM GRAPH// |
| 5D | NR OR | 4-5 w | GRO | WGHT | NR | - 1 | sig | p<0.05 | P | W O | N R | >400/ - <~700/ | N R | N R | g | N R | 1. RVALUE/F ROM GRAPH// |
| 6D | NR OR | 1-3 w | GRO | WGHT | NR | - 1 | mult | p<0.05 | P | W O | N R | >100/ - <500/ | N R | N R | g | N R | 1. RVALUE/ FROM GRAPH// |
| 6D | NR OR | 4-5 w | GRO | WGHT | N | - 1 | sig | p<0.05 | P | W O | N R | ~400/ - <~500/ | N R | N R | g | N R | 1. RVALUE/ FROM GRAPH// |
| 7D | NR OR | 1-5 w | GRO | WGHT | NR | - 1 | sig | p<0.05 | P | W O | N R | >0/ - <~400/ | N R | N R | g | N R | 1. RVALUE/ FROM GRAPH// |

Example 2. Non-analyzed Data



Terretox Results Coding for Example 2

| ID | SMP # UNITS | OBSERV DUR | EFFECT | MEAS MENT | END PNT | R | STAT | LEVEL | P | SIT E R | OBSERVED RESPONSE VALUE/UNIT | | | | | D W % | REMARK |
|----|----------------|---------------|--------|--------------|------------|----|------|-------|---|---------------|------------------------------|---------------|----|-------|------|-------------|-------------------------|
| | | | | | | | | | | | MEAN | RANGE | SM | VALUE | UNIT | | |
| 1C | NR OR | 1-5 w | GRO | WGHT | NR | -1 | NR | NR | P | W O | NR | ~100/ - ~700/ | NR | NR | g | NR | 1. RVALUE/ FROM GRAPH// |
| 8R | NR OR | 1-5 w | GRO | WGHT | NR | -1 | NR | NR | P | W O | NR | ~100/ - ~700/ | NR | NR | g | NR | 1. RVALUE/ FROM GRAPH// |

Species Data

Data may be reported for an individual species as well as for a community or population. For example an author may report that biomass for an earthworm, *Eisenia fetida*, has decreased and that the invertebrate population biomass has increased. Report the measurements and endpoints as reported by the author for both the individual species and the species group.

Dose ID & Dose No

This is the same Dose ID and Number as recorded for each treatment level under the in Exposure Information. Transcribe the ID and Dose Number for each treatment level.

Sample Number (SMP#) and / Units

The sample number reflects the sample size (e.g., 10 embryos) that the observation or response value is based on at each exposure level. For endpoints based on calculations (e.g. LD50, NOEL, etc.) rather than individual dose measurements, the sample number will be coded as 'NR'. Code 'NR' if no information about the observed sample has been reported.

Sample units correspond to the sample number; i.e., the unit on which the measurement or endpoint is based (see Appendix Q for applicable codes). Code 'NR' if the sample unit is not reported.

EXAMPLE: For a sample size of 190 eggs, the sample unit is eggs (EG); therefore, if the effect measurement is HTCH, and the observation response value is 90%, then 90% of 190 eggs hatched.

Note: For generational studies and measurements based on the progeny, note F1, F2, etc. in the sample units field.

Note: If a sample number is not provided, but a "unit" is, always enter the unit in the sample units field.

Note: A FieldN test scenario involves exposing plots or sample areas, in addition to specific test organisms. Usually the number of exposed organisms is unknown. The number of plots or sample areas is coded as '#/EU' (the number of experimental units) in Results Information rather than in the Exposure Information Sample Number field. See Table 6 for coding examples

Observation Time Duration (OBSRV DUR)

The Observation Duration reported includes exposure time plus any additional days up to the time at which the response to the toxicant was observed. It does not include pre-treatment time. In the example in Table 4, the observation was made on day 11; therefore, the observation duration time is 9D. If the observation time is not reported or unable to be explicitly determined, code as less than

or equal to (\leq) the exposure duration. NR should not be coded in this data field.

Observations during the pretreatment time are reported as negative values. For the Table 4 example, the observation time for a pretreatment sample collected on day 2 of the pretreatment period would be recorded as -1D. Report as '-x' any pretreatment response observations for which time is unknown.

In some cases a biological time is used, such as an observation time reported as "until hatch", "growing season" or "after the nth egg has been laid". Use the code from Appendix I that best describes the author's words in duration units data field. 'NA' will be coded as the duration mean value for all biological time durations (e.g. OBSERVATION MEAN: NA, OBSERVATION UNIT: HT; corresponds to an until hatch duration), unless a numeric value is substituted (e.g. OBSERVATION MEAN: 2, OBSERVATION DOSE: HV; corresponds to a 2 harvests duration). However, references to time such as "observed at end of the study period" are not coded; such papers are rejected as having no observation duration.

Note: In test scenarios where incubation times are reported, e.g., enzyme fixation assays, be careful to report the toxicant exposure time *not* the assay incubation time.

Note: In test scenarios that involve generational studies, the observation duration times are reported from the time the parents were exposed. For example the parents were exposed for 10 months prior to mating, and the progeny was born 2 months later, the observation duration for both the adult REP PROG effect and for the juvenile DVP ABNL is 12 months. The exposure duration would be the same for both - 10 months. The only difference between the two effects is in the sample unit. The sample unit for the adult effect would be 'AD' and for the juveniles it would be 'F1'.

Table 6 . Results Information Coding Examples

| Dose ID & No. | SMP # UNIT | OBS DUR | EFCT | MEAS | END PT | R | S T A T | L V L | P | S I T E | OBSERV.RESP VALUE/UNIT X Range SM Value Unit | DW % | % L P D | R A N K | R N | REMARKS |
|--|------------|---------|------|------|--------|----|---------|-------|-----|---------|---|------|---------|---------|-----|--|
| 1C | 10 F | 5h | MOR | MDTH | NR | -1 | N R | N R | N R | NR | 11.5 SD 7.8 d | NR | N R | - | 1 | 1 RVALUE/ from graph// 2 MSMT/ asymptotic level// |
| 1C | " " | " | ACC | RSDE | " | -1 | " | " | " | WO | 1245 ug/g | W 25 | 42 | - | 2 | |
| 3V | " " | " | MOR | MDTH | " | -1 | " | " | " | NR | 15.8 SD 5.9 d | NR | N R | - | 1 | |
| 4D | " " | " | " | " | " | -1 | " | " | " | " | 12.8 SD 7.6 d | " | " | - | 1 | |
| 5D | " " | " | " | " | " | -1 | " | " | " | " | 15.6 SD 5.5 d | " | " | - | 1 | |
| 5D | " " | " | ACC | RSDE | " | -1 | " | " | " | WO | 1459 ug/g | W 33 | 44 | - | 2 | |
| 6D | " " | " | MOR | MDTH | " | -1 | " | " | " | NR | 16.6 SD 8.0 d | NR | N R | - | 1 | |
| | | | | | | | | | | | | | | | | |
| 1C | 10 EU | 1-5d | REP | PROG | NR | -1 | N R | N R | N R | NR | 680 (645-690) eg/d | NR | N R | - | N R | |
| 2C | " | " | " | " | " | -1 | " | " | " | " | 983 (825-1012) eg/d | " | " | - | N R | |
| 3V | " | " | " | " | " | -1 | " | " | " | " | 259 (243-272) eg/d | " | " | - | N R | |
| 4D | " | " | " | " | " | -1 | S I G | < 0.5 | P | " | 246 (232-257) eg/d | " | " | - | N R | |
| 5D | " | " | " | " | " | -1 | " | " | " | " | 255 (242-267) eg/d | " | " | - | N R | |
| | | | | | | | | | | | | | | | | |
| Data for REP/PROG would be continued for dose levels 3 and 5 | | | | | | | | | | | | | | | | |
| 8E | NR NR | 20d | MOR | MORT | LD50 | -1 | N R | N R | P | NR | 9.8 (5.6-11.2) d | NR | N R | - | 1 | |

When coding the endpoints LTxx or ETxx, which are based on the time it takes to get a XX% response, code the associated effect response time and not the total exposure time in the OBSERVED DURATION field.

Example 1: A test with earthworms is run for 10 hours, but it takes 6 hours for the "T1/2" duration which is the time to take 50% to burrow. The author reported T1/2 endpoint is similar to an ET50 endpoint, therefore a reviewer assigned ET50 is coded.

Code: Assigned Endpoint: R Endpoint: ET50 Effect: BEH Measurement: BBBH Observation
Duration: 6 h

Example 2: A 4 week study is run with quail and reports mortality. The author reports the LT50 value at 10 mg/kg, 20 mg/kg and 30 mg/kg as 3.5 weeks, 2 weeks and 1 week, respectively.

Code:

Assigned Endpoint: P Endpoint: LT50 Effect: MOR Measurement: MORT Duration: 3.5 wk

Concentration: 10 mg/kg

Assigned Endpoint: P Endpoint: LT50 Effect: MOR Measurement: MORT Duration: 2 wk

Concentration: 20 mg/kg

Assigned Endpoint: P Endpoint: LT50 Effect: MOR Measurement: MORT Duration: 1 wk

Concentration: 30 mg/kg

Effect

Ecotoxicology is the study of the "toxic effects of natural or artificial substances on living organisms (e.g. fish, birds, plants) ..." The effects may manifest at various levels of organization from sub-cellular through individual organisms to communities and ecosystems. Effects may be both positive and adverse; toxicology focuses on the adverse effects. Adverse effects include short-term and long-term lethality and sub-lethal effects such as changes in behavior, growth, development, reproduction, uptake and elimination, and tissue structure. (Rand 1995)

In TERRETOX, effect groups include accumulation, behavior, biochemistry, cellular, growth, mortality, physiology, population, reproduction and ecosystem (see Appendix R for definitions).

Within each effect group, the observed effect must be quantified in a reproducible way. In TERRETOX, two mechanisms are used to represent the observed effect: measurements and endpoints. Measurements include quantitative observations that describe and evaluate biological responses to toxicants, while endpoints are based on calculations derived from statistical analysis of the observations. Therefore, while measurements are direct biological observations, endpoints provide a statistical comparison of responses to toxicants. Coding criteria for each of these mechanisms is described below, directly following the General Notes section. The 'General Notes' section provides guidelines for extracting effect/ measurement data from the publication.

Effect Measurement (MEASMENT)

Generally, "measures" or "measurements" are variables used to aid in the interpretation of the degree of response to a toxicant by an organism. For example, measures of behavioral effects in TERRETOX include general behavioral changes (BEH GBHV), changes in feeding activity (FDB FDNG), and stimulus avoidance (AVO STIM). Appendix S lists the measurements currently used for each of the effects in the TERRETOX database. If more than one organism is measured in either an artificial (pot, cage, aquaria, etc.) or natural population (field, water body, etc.), reviewing staff will code the effect and measurement as POP BMAS (population biomass). For instances where there is an individual biomass (one organism, artificial or natural), reviewing staff will code GRO BMAS (growth biomass).

Endpoint/Result Set (R)/ Stat/Level/Assigned (PR) (ENDPT/STAT/Level/P or R)

Endpoint (ENDPT)

An endpoint is a value derived from statistical analysis or calculation of a specific measurement, or series of measurements, made during the test. Endpoints may be classified as measurement endpoints or assessment endpoints. Assessment endpoints refer to environmental parameters such as population, community or ecosystem measurements, e.g., growth rates or sustainable yields. Measurement endpoints refer to specific variables that are used to evaluate the assessment endpoints, such as diversity or evenness. (Hoffman et.al. 1995; US EPA 1996)

The ECOTOX databases utilize assessment and measurement endpoints which quantitatively represent the response(s) of a given individual, population, or community to the effects of a toxic agent. Appendix T lists and defines endpoints used in TERRETOX. For each endpoint, effect and measurement must also be coded. Refer to Table 6, Results Information, for coding examples.

For some endpoints, linkage to an exposure dose, and therefore an Exposure Dose Number, is

especially important. These endpoints include BAFs or time associated endpoints such as LTxx and ETxx and TKNO. However, endpoints that are not linked to a specific concentration, e.g., LDxx, are not associated with an exposure number because the observed result is based on a calculated rather than an observed dose. These endpoints are linked with a placeholder Dose ID and Dose Number. The linkage is noted by E in the DOSE ID data field and the associated DOSE NO. in the EXPOSURE information. Refer to Table 5, Results Information, for coding examples.

In contrast, NOEC/NOELs and LOEC/LOELs are the endpoints used to represent a statistically significant range within the tested concentrations. The NOEC/NOEL is the highest tested concentration having no statistically significant adverse effect and the LOEC/LOEL is the lowest tested concentration having a statistically significant adverse effect. (Rand 1995) NOEC/NOELs and LOEC/LOELs are also linked by the placeholder exposure number, as indicated in the previous paragraph.

For endpoints of ETXX or LTXX, code both the observation duration and the observed response value with the same values.

Result Set (R)

This field is used to link effects and endpoints together for data output display. The default entry for this field is -1. Enter positive integers for each specific effect/endpoint linkage. For example, a mortality data table and an LD50 as well as a body weight growth table and an EC50 are reported. Code all of the mortality data table and the LD50 with a '1' in the R data field and code '2' in the R data field for the growth and EC50 data. If there are endpoints without data or vice versa, code '-1' in the R data field. See Table 6, for examples.

Significance (STAT)

The significance or STAT data field is coded with SIG or NOSIG if the author has presented statistical analysis of the test result as compared to the controls. As a general rule, if statistics are presented in the publication, assume that the exposure treatments were compared to the control. Statistical tests that measure differences between treatments are not coded. See Tables 7 and 8 for coding examples.

When statistical comparisons are presented for multiple controls (e.g., statistics in relation to a standard and solvent control), both sets of results are coded. In these instances, note the specific type of control used in the statistical analysis in the Remarks section.

The STAT data field is coded as "NR" for records having an endpoint of LCxx, ECxx, LTxx, BAF, ETxx, ICxx, LDxx. For NOEC/NOEL, LOEC/LOEL and statistically analyzed effects results without endpoints, code the significance as reported by the author(s), or 'NR' if statistical results are not presented in the publication.

If the author states that there is a 'statistically significant' increase or decrease in an observed effect (whether or not they report the statistical method used) but does not report a level of statistical significance level or identify a method of statistical analysis, code 'SIG' or 'NOSIG' and 'NR' in LEVEL field. If the author states there is a significant increase or decrease in an observed effect but does not say it is "statistically significant," code 'NR' in the STAT data field.

Note: For concurrent control results, there is no STAT or LEVEL defined. Statistical significance is compared to the control values.

Note: Coding statistics from least square differences (LSD).

If a paper presents data in the following format, determine the statistical significance by the following calculation.

| Dose | Response |
|-----------|----------|
| Control 1 | 2.4 |
| Dose 2 | 4.6 |
| Dose 3 | 4.8 |
| Dose 4 | 6.7 |
| LSD(0.05) | 1.1 |

Subtract or add the LSD value (1.1) to the control value to get the lowest value that is significantly different. In this example, anything above 3.5 or below 1.3 is a significant value at the $p=0.05$ level. Therefore, all doses are significantly different from the control because they are all greater than 3.5 (Pers. Comm., R. Regal, UMD Statistics Dept., 1999).

Level

The level of significance (e.g. test statistic) is coded when the author has reported statistical analysis in the test result. Terminology for significance level may be presented as: $p =$; $p <$ or alpha value; ². The terminology is equivalent and is generally in the range of 0.001 to 0.10. See Tables 7 & 8 for coding examples.

The LEVEL data field is coded as reported by the author. If endpoints of LCxx, ECxx, LTxx, BAF, ETxx, ICxx, Ldxx report confidence intervals/limits, etc., report the significance level in the LEVEL field, e.g., 95% CI is coded as 0.05.

Paper/Reviewer Assigned Data (P or R)

The PR data field is used to identify the source of the effect or endpoint information. If the effect or endpoint was reported by the author in the publication a 'P' is coded in the PR data field; if the effect or endpoint was assigned by the reviewer, an 'R' is coded. See Tables 7 & 8 for coding examples. Endpoints calculated by the author must be specifically identified, i.e., LD50, LT50 or NOEL/NOEC (see Appendix T for endpoint codes and definitions).

Reviewers will follow these guidelines in *assigning* endpoints:

1. BY DEFINITION: If the author does not actually state that the value is an LD50 but states that "concentration x is the dose estimated to be lethal to 50% of the test organisms", the reviewer should code this as an LD50 endpoint because the author *defines* the LD50. Such a designation is accompanied by noting 'R' in the PR data field.
2. When the author provides text which identifies a value as the "highest tested concentration having no statistically significant adverse effect", the reviewer should code this as a NOEL/NOEC ; the "lowest tested concentration having a statistically significant adverse effect" is coded as a LOEL/LOEC . In both cases, the PR field will be coded as 'R' to reflect reviewer assignment of an endpoint. Because LOEL/NOEL values are assigned under very specific experimental and statistical conditions, TERRETOX reviewers will be assigning complementary NOELs or LOELs only when the author assigns either a LOEL or NOEL.
3. When the author provides statistical information, which designates concentrations as significantly different from the control, the reviewer will code this information as SIG or NOSIG. The reviewer will also report the level of significance in the LEVEL data p-value in the p-value field.

Table 7. Coding Statistical and Endpoint Data Directly from a Table.

"The data from our experiments is shown in the following table."

| # | Conc ug/g | Survival % | Stat sig p<0.05 | Calc Endpt |
|----|-----------|------------|-----------------|------------|
| C1 | 0 | 97 | NR | NR |
| D2 | 7 | 97 | NOSIG | NR |
| D3 | 15 | 75 | NOSIG | NOEL |
| D4 | 30 | 26 | SIG | LOEL |
| D5 | 50 | 0 | SIG | NR |

In this example, the raw data table is coded as follows:

| <u>DOSE</u> | <u>EFFECT</u> | <u>MEASMENT</u> | <u>ENDPT</u> | <u>R</u> | <u>STAT</u> | <u>LEVEL</u> | <u>P R</u> |
|-------------|---------------|-----------------|--------------|----------|-------------|--------------|------------|
| C1 | MOR | SURV | NR | 1 | NR | NR | P |
| D2 | MOR | SURV | NR | 1 | NOSIG | p<0.05 | P |
| D3 | MOR | SURV | NR | 1 | NOSIG | p<0.05 | P |
| D4 | MOR | SURV | NR | 1 | SIG | p<0.05 | P |
| D5 | MOR | SURV | NR | 1 | SIG | p<0.05 | P |
| E6 | MOR | SURV | NOEL | 1 | NOSIG | p<0.05 | P |
| E6 | MOR | SURV | LOEL | 1 | SIG | p<0.05 | P |

Table 8 . Coding Statistical Data Directly from a Table with a Reviewer Assigned Endpoint.

"The data from our experiments, in Table Z, shows that the concentration that had no observable effect on mortality was 7 ug/g."

Table Z: Mortality of *Eisenia fetida* to copper

| # | Conc ug/g | Survival % |
|----|-----------|------------|
| C1 | 0 | 97 |
| D2 | 7 | 97 |
| D3 | 15 | 75 |
| D4 | 30 | 26 |
| D5 | 50 | 0 |

In this example, the table is coded as follows:

| DOSE | EFFECT | MEASMENT | ENDPT | R | STAT | LEVEL | P R |
|------|--------|----------|-------|---|-------|-------|-----|
| C1 | MOR | SURV | NR | 1 | NR | NR | P |
| D2 | MOR | SURV | NR | 1 | NR | NR | P |
| D3 | MOR | SURV | NR | 1 | NR | NR | P |
| D4 | MOR | SURV | NR | 1 | NR | NR | P |
| D5 | MOR | SURV | NR | 1 | NR | NR | P |
| E6 | MOR | SURV | NOEL | 1 | NOSIG | NR | R |
| E6 | MOR | SURV | LOEL | 1 | SIG | NR | R |

Standard methods recommend that when determining a NOEL/LOEL, at least three exposure concentrations be used (Menzer 1994 at 1406);. If the *author* uses only one exposure concentration AND assigns a NOEL/LOEL or SIG/NOSIG result, a Remark noting "only conc tested" will be coded.

Response Site (SITE)

The specific site at which an effect measurement was observed is coded in the SITE data field, e.g. for residues (RSDE) recorded in the "liver," enter 'LI' in the SITE data field (see Appendix U for applicable codes). Response site is valid entry for GRO, AEG, CEL, PHY, DVP, GEN, REP, HIS, ENZ, BCM, HRM, INJ, MPH and ACC effect groups (see Appendix S for effect group and measurement codes). If a response site is not reported or not applicable, e.g. mortality, behavioral effects, code the site as Not Reported (NR).

If data are presented without statistical analysis in a graph or figure, results for each measurement are combined by response site.

Observed Response Value/Unit: , Mean, Range, Statistical Method (SM), Value, Unit (Mean, Range, SM, Value, Unit)

Enter the greater than (>), less than (<), minus (-) or approximation (~) symbols, if reported, as used by the author(s) to describe the response value preceding the MEAN or RANGE data field entries.

Report the mean or single observed response value, as reported in the publication, in the MEAN data field. When individual response values are reported along with a sum of all values, report each individual response value as well as the sum/total value on separate result lines.

Report the range or confidence (or fiducial) intervals (or limits) of the response value in the RANGE data field. The type of data stored in the RANGE data field will be identified in the SM data field (e.g., Data reported as a range (with a mean) or confidence interval (with an endpoint) will be

specifically identified in the SM field. It is also assumed that the confidence interval is calculated at 95% and is noted in the LEVEL data field.

When the measurement unit includes a standard deviation (SD) or standard error (SE), specifically identify these types of ranges in the SM data field. Report the numeric value of the standard deviation or standard error in the VALUE data field.

Report the measurement unit which corresponds to the MEAN and/or RANGE entry in the UNIT data field (see Appendix N for standard units).

Refer to Table 6 in Results Information for coded examples. Table 6a. provides a standard deviation example, Table 6b. provides an example of a range, and Table 6c. provides a confidence interval example.

Dry or Wet Weight (DW%)

Record whether the residue/bioconcentration/bioaccumulation or growth data are reported on a dry or wet weight basis in the DW% data field. If percent moisture is reported, record the percentage value also, e.g. W75%.

Percent Lipid (%LPD)

If percent lipid information is provided in the publication, record as a % value in the %LPD data field. If the data are not reported in the publication, code as 'NR'.

Rank (RANK)

Following evaluation by EcoSSL (Ecological Soil Screening Levels) Task Group reviewers, this field will be marked for each test result for each publication to indicate the Evaluation Criteria Score and selected benchmark value ranking for determining the EcoSSL value. Prior to this, leave this data field blank.

Remark Number/Remarks (RN/REMARKS)

When there are remarks for a specific test, the REMARKS field as well as the Remark Number (RN) field, will be used. The Remark Number field is used to link the remarks associated with each specific test result. Each unique Remark is assigned a Remarks Number and only one Remark Number is used per result entry. Use a unique Remark Number for each section of the database, i.e., do not carry over Remarks or Remark Numbers from the Exposure Information to the Results

Information sections. Remarks are preceded by the Remarks Number and identified by the field abbreviation listed in Appendix EE. Refer to Table 6 for coding examples.

General Coding Information

Q. What is encoded from a publication?

A. All quantitative data are encoded from the publication. Each data point from tables, text and graphs is coded. Graphical data may be coded as ranges (1 result for the control and 1 result for all of the doses), unless statistical analysis is performed. Graphed data is reported by using <, > or ~ values. These values must be the values noted by the axis marks from the graph. If duplicate results are reported in text and tabular format, note in the margin of the paper that the text information was coded from Table N. Non-quantitative data are noted in the general remarks section.

Q. Are abstractors allowed to interpret results from publications?

A. All information from a paper is abstracted using the author's terminology and numeric values. Exceptions to this include the expansion of exponential numbers and when the author's "words" match the standard definition effects and endpoints. If an endpoint is "interpreted" by an abstractor, it is noted by an 'R' in the ASSIGNED P/R data field.

Q. How and why are comments made?

A. In general, comments are used to better define or capture the researcher's intent. THESE ARE USED SPARINGLY. Comments are linked to coded fields by an identifier in the appropriate comments field (Organism, Exposure, Soil, General or Results information/remarks data fields). For example, a RESPONSE VALUE comment of median LC50 is located in Result remarks data field as Rvalue/median LC50//. Some comments are not linked to a specific data field (i.e. exposure temperature or in vitro studies). These comments are also noted in the appropriate comments field (i.e. exposure temperature in Exposure information and in vitro in the general remarks data fields).

Q. Is anything written in the original paper by the data abstractors?

A. Abstractors should note any comments about abstraction in the margins or on the tables/graphs of the original paper. This would include the Test ID Number for each unique test design, reason for data not being coded, LD50s outside of confidence intervals, errors between text and tables, or other anomalies.

Q. What happens if an endpoint is outside the confidence interval/limit or text and tabular or text and abstract data points differ?

A. The abstractor encodes only the endpoint value and notes that the range was not coded in the original publication. Textual information is used over all other data, unless the value is noted in another section of the paper. Then, the most frequent value is encoded.

6. Supporting Materials

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